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The Effect of Estrogen on the Incidence of Mammary and Pituitary Tumors in Hybrid Mice*†

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The influence of administered estrogenic hormones on the incidence of mammary tumors of male mice of several strains has been studied since the initial observations of Lacassagne (18). Male mice from strains in which the females have a high incidence of mammary tumors will show a high incidence of such tumors after adequate treatment with estrogen. Mice from strains normally showing few or no mammary tumors infrequently develop such tumors after injections of estrogen which would result in mammary neoplasia in male mice of the susceptible strains (9, 19, 21). These observations, although demonstrating the essential role of estrogenic hormones as originally stated by Loeb (20), also indicated the existence of other factors determining the susceptibility to tumors. One of these factors is transmitted from mother to offspring and is apparently transmitted by the milk (2).

The mammary glands of old female mice from strains of varying susceptibilities to mammary carcinogenesis showed characteristic differences that could be associated with the tendency to undergo malignant changes (7, 10, 25). A similar differentiation of the mammary parenchyma of mice from strains susceptible or resistant to mammary tumors occurred in males that had received estrogenic hormones. In those experiments in which estrogens were administered in similar amounts to mice of the high- and low-tumor strains, a comparable internal hormonal environment of all of the glands presumably existed. At least two influences in addition to the hormonal environment still exist which might affect the varying responses of the mammary tissue, namely, a genetic factor and the "milk factor." A uniform genetic constitution may be maintained, in the absence of the sex-linked factors, and the milk factor may be varied by reciprocal crosses of females and males of a high- and low-tumor strain. Such a technic was employed in the following experiments to study the effect of estrogen on the structure of the mammary glands and on the incidence of mammary carcinomas of genetically similar animals.

Another neoplasm appearing in estrogen-treated mice, which in this laboratory has been strain-limited, involves the pituitary gland (12). The present experiments were designed so that the possibility of a genetic transmission of the tendency to develop this neoplasm might be determined. At the same time the possibility of a maternal transmission of the tendency to develop hypophysial tumors could be ascertained.

MATERIALS AND METHODS

Male and female mice of the CBA strain [1] were mated with males and females of the C57 strain. The offspring of the CBA females x C57 males were designated CC_2 and those of the C57 females x CBA males, CC_1. The parent mice as well as other mice of the same strain were observed throughout their lives to establish an incidence of tumors under the conditions prevalent in the laboratory. Fifty-two mice of the CC_2 and 53 mice of the CC_1 groups received weekly subcutaneous injections of 16.6 or 50 μgm. of estradiol benzoate [2] starting at ages ranging from 28 days to 56 days, and continuing throughout life. Only 7 of the CC_1 mice received the larger weekly dose. A similar number of mice were retained as untreated controls. The untreated females were usually caged with the males and as the young were removed at birth they were almost continually pregnant (1). The numbers of males and females in the control and experimental groups are listed in Table I.

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[1] The mice of the CBA and C57 strains were obtained from Dr. L. C. Strong. The former stock was developed in his laboratory (23) and the C57 stock obtained by him from Dr. H. J. Bagg.

[2] The estradiol benzoate was generously supplied by Dr. E. Schwenk of the Schering Corporation.

All mice were kept under comparable conditions in the same room of an air-conditioned building. Food (Purina Fox Chow) and water were available at all times. The mice were removed when tumors developed or when their general condition indicated that death was imminent. A few animals died but as they were checked once or twice daily a satisfactory postmortem examination usually could be made.

At autopsy the mammary glands were removed on the skin and prepared as gross mounts using a procedure previously described (8). The tumors and other interesting areas of mammary tissue were removed and sectioned for microscopic study. The genital tissues, adrenals, and hypophyses of most of the mice were preserved and studied microscopically. In some animals the kidneys, spleen, liver, thyroid, and other tissues also were preserved. The skeletal tissues were examined, many of the femurs were broken to determine breaking strength, and studied to determine the extent of osseous change.

(Table I). The mice which did not have hypophysial tumors survived for periods averaging over 100 days less than those in which such tumors appeared.

The incidence of hypophysial tumors was not only greater in males than in the females but the tumors appeared at relatively earlier ages, and frequently attained greater sizes. The incidence of such tumors was greater in both male and female mice of the CC_1 group, that is, in mice with the C57 maternal parent. The difference is not statistically significant, however, when the males are compared. The male mice of the CC_1 group survived an average of over 30 days longer than those of the CC_2 group in which the maternal parents were from the CBA strain. Of the males and females the proportion dying with hypophysial tumors tended to increase with advancing age. This tendency was especially apparent for the female mice (Figs. 1 and 2). The tendency for the lower incidence of hypophysial tumors in the hybrid females in contrast to the males is significant. The same tendency for a

TABLE I: THE INCIDENCE OF HYPOPHYSIAL TUMORS IN HYBRID MICE RECEIVING ESTROGENS

Mice	Sex	Total number	With pituitary tumors		Age of tumors		Average age at death Non-tumor, days
			No.	Per cent	Average, days	Range, days	
CC_2	F	28	9	32	562	431-641	442
CC_1	F	30	16	53	599	492-626	419
CC_2	M	24	18	75	480	326-671	360
CC_1	M	23	19	83	513	380-593	320
Totals		105	61	58			

OBSERVATIONS

Pituitary tumors.—The pituitary glands of mice of most strains hypertrophy slightly following the injection of estrogens. Animals of the C57 strain, which survived 300 days or more and received 16.6 μgm. of estradiol benzoate weekly, frequently had hypophysial tumors (12). Such tumors rarely appeared in mice of other strains subjected to similar treatment in this laboratory.

Pituitary glands weighing in excess of 12 mgm. were considered to be tumors. This is an arbitrary figure representing a hypertrophy of over 6 times the usual hypophysial weight. A number of the smaller pituitary tumors weighing over 12 mgm. were probably glands showing a generalized hypertrophy of the chromophobic cells as previously reported for the C57 strain. The larger tumors were adenomas of nongranular cells. The adenomas also resembled those described in estrogen-treated mice of the C57 strain.

The incidence of hypophysial tumors in the hybrid offspring of the C57 and CBA strains used in these experiments was 58 per cent, 61 of the 105 mice dying with pituitary glands weighing over 12 mgm. Forty-one per cent of the 58 females had pituitary glands exceeding 12 mgm., and 79 per cent of the males had similar tumors at a somewhat earlier average age

lower incidence of tumorous changes of the pituitary glands was also apparent in estrogen-treated mice of the C57 strain (12).

Mammary carcinoma.—Among the estrogen-treated animals mammary carcinoma appeared only in mice of the CC_2 group (Figs. 3 and 4). Approximately 60 per cent of these mice showed mammary cancer at autopsy. The cancers appeared in mice at ages ranging from 195 to 671 days. Two or more large mammary cancers were found in 3 mice. In other mice small nodules, to be described later, were present in addition to the large cancers, and some of these nodules were definitely early carcinomas. The mice were usually killed when the cancers attained a diameter of 1 to 2 cm. More animals would have developed multiple tumors had a longer survival been permitted.

The cancers resembled histologically those arising in mice of the CBA strain. Some of them contained dark hemorrhagic cysts. These tumors were usually adenocarcinomas with many small alveolar groups of epithelial cells in delicate connective tissue septa and large cysts filled with blood or a hemosidrin-containing fluid. The tendency for invasion of the surrounding tissues was not usually particularly evident but no definite encapsulation of the tumors was apparent. The impression was obtained that a progressive malignant

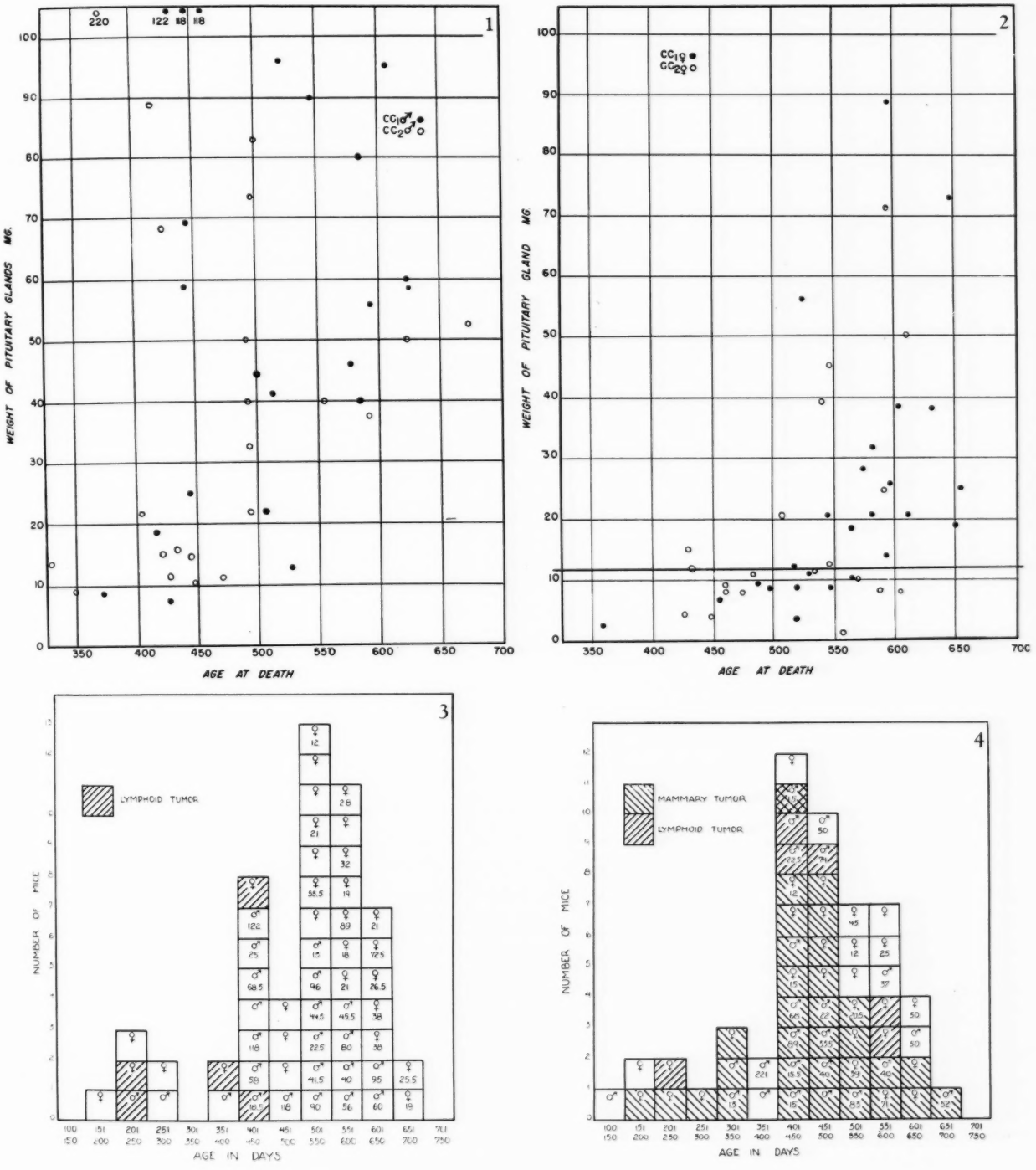


FIG. 1.—The age distribution and weights of the hypophysial tumors in the hybrid male mice.

FIG. 2.—The age distribution and weight of the hypophysial tumors in the hybrid female mice.

FIG. 3.—The age distribution of mammary and lymphoid tumors in the estrogen-treated hybrid mice of the CC_1 group. The figures in the squares are the weights of the hypophysial tumors.

FIG. 4.—The age distribution of mammary and lymphoid tumors in the estrogen-treated hybrid mice of the CC_2 group. The weights of the hypophysial tumors are given in the squares.

transformation of adjacent noncancerous mammary tissue was also adding to the size of the neoplasia. Other tumors consisted of massive cords of epithelial cells and a delicate stroma. Some tumors, predominantly composed of cords of cells, showed areas of alveolar tissue indicating mixed or transitional types of organization. A few intraductal papillary carcinomas were also observed and one squamous cell epithelioma.

In addition many small nodules were removed from the partially dissected glands and sectioned for microscopic study (Figs. 5 to 9). Many of these nodules were histologically identical with the larger carcinomas of the various types mentioned above. These small tumors also appeared to be enlarging by a progressive involvement of surrounding tissues as well as by direct invasion (Figs. 19 and 20). A few of these nodules showed histologically progressive malignant degeneration from without inward, the periphery appearing adenomatous with more atypical tissue medially located.

TABLE II: INCIDENCE OF MAMMARY AND LYMPHOID TUMORS IN THE HYBRID MICE RECEIVING ESTRADIOL BENZOATE

			Mammary tumors		Lymphoid tumors	
Group	Sex	Number	Number	Per cent	Number	Per cent
CC_2	F	28	17	60	3	10
	M	24	14	58	4	17
Total		52	31	59	7	
CC_1	F	30	0	0	3	15
	M	23	0	0	2	9
Total		53			5	

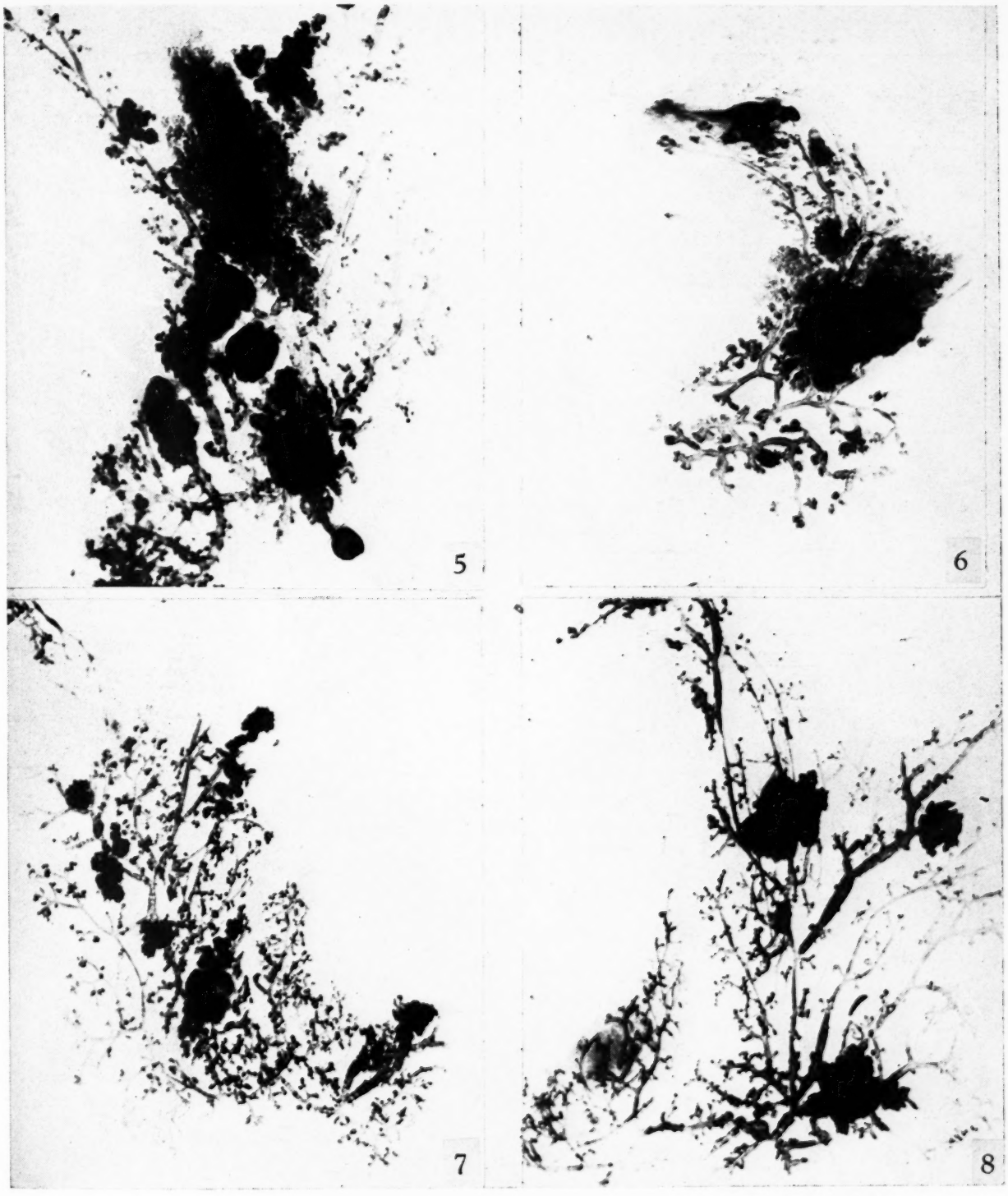
Not only did the incidence of mammary tumors of the estrogen-treated CC_1 and CC_2 groups differ greatly but the general morphology of the mammary glands also varied in an equally striking manner. The amounts of estrogen used resulted in an extensive proliferation of the mammary ducts of the males and females of both groups. Some of the glands of mice of both groups consisted largely of these branching ducts (Figs. 5 and 9). In other mice the glands contained many more small terminal ducts or alveoli (Figs. 7 and 10). The glands of other mice contained uniformly distributed lobules of alveoli (Fig. 12) while others were less completely developed (Fig. 11). Grossly the glands resembled those of mice at varying stages of sexual maturity or pregnancy. No significant variation existed between the two groups in the above characteristics of the mammary glands.

Another common characteristic of the glands of the two groups was the presence in the ducts of a peculiar material apparent in both the intact and in fixed and stained glands (Figs. 13 and 15). This material existed in the ducts in a solid or semisolid state. The concretion-like masses were found in groups and in chains giving the ducts a beaded appearance, or these masses were isolated (Figs. 13 and 15). In some instances they were sufficiently large to distend the ducts to several times their usual size. The intraductal masses were found in the glands of all of the estrogen-treated mice but rarely in the untreated animals.

The mammary glands of the estrogen-treated mice of the CC_1 group were usually uniform in structure throughout (Figs. 9 to 12). On the other hand the individual glands of mice of the CC_2 group almost always showed a characteristic lack of uniformity in different areas, whether or not the animals had mammary carcinoma (Figs. 5 to 8). Nodules of denser epithelial tissue were present at random points throughout the glands in all except one mouse. The gross structure of these nodules studied in the stained dissected glands varied discernibly. Some of the nodules were composed of groups of large alveoli, others of well circumscribed clusters of small alveolar structures (Fig. 13) and others of indefinite epithelial structures and fibrous tissue. Some of the nodules were composed of a dense but diffuse overgrowth of small ducts or alveolar tissues and were called diffuse adenomatous overgrowths (Figs. 5, 6, 14). Other nodules were so dense that their structure could not be determined from the preparations of the dissected glands.

Microscopic examination of sections of the glands revealed other details of the nodules described above as determined by observation of the dissected and stained preparations. Some of the nodules were small histologically malignant tumors of the adenocarcinomatous or medullary types. These small carcinomas often appeared in areas of the glands showing an adenomatous hyperplasia. Other nodules contained large ducts filled with the concretion-like bodies and many smaller alveolar structures (Figs. 18 and 21). The epithelium on some of the ducts was completely lacking around the concretions. In other ducts epithelial cells surrounded the concretions or even filled the greater part of the ducts. Other nodules or parts of nodules were apparently regressing. The nuclei of the epithelial cells appeared pyknotic and the cytoplasm was pale or cellular detail was not evident. The stroma of such nodules was infiltrated with mononuclear cells and consisted of coarse collagenous fibers. Other nodules consisted of ducts containing variable numbers of cysts lined by stratified squamous and cornified epithelium. The stroma of such nodules was usually dense and fibrous.

The diffuse adenomatous overgrowths consisted of many small branching ducts and small alveolar processes lined by a single layer of cells (Figs. 16 and 17). The stroma consisted of a loose vascular connective tissue. These overgrowths arose from a single area along a mammary duct and extended through the subcutaneous tissues and occasionally surrounded other mammary ducts. Many mitotic figures appeared



Photographs of one of the second pair of mammary glands from each of 4 mice of the CC_2 group which had received 16.6 μgm. estradiol benzoate weekly.

FIG. 5.—From a female which had received 0.950 mgm. in 405 days, starting at 50 days. The glands consist of ducts and small branches and contain several nodules and one diffuse adenomatous growth. This animal had a mammary adenocarcinoma in the interscapular region, a hypophysis weighing 9.5 mgm., and brown degeneration (Stage 2).

FIG. 6.—Two glands from a male which had received 0.916 mgm. in 391 days, starting at 34 days. A large diffuse overgrowth extends throughout the central portion of the gland. The second gland (small spot to left) is rudimentary as in an untreated animal. This mouse had a large infiltrating mediastinal lymphosarcoma, a hypophysis weighing 11.5 mgm. and brown degeneration (Stage 3).

FIG. 7.—From a female which had received 1.066 mgm. in 452 days, starting at 29 days. Many small ducts were present. Several small nodules are seen at various places. This mouse had a medullary carcinoma in one of the glands of the 4th pair, a large pituitary gland, brown degeneration (Stage 2) and early adenomatous change of the adrenal gland.

FIG. 8.—From a female which had received 0.966 mgm. in 409 days, starting at 50 days. Three large nodules appeared on the branching ducts. The mouse had a medullary carcinoma of one of the glands of the 5th pair, a hypophysis weighing 9 mgm., brown degeneration (Stage 2), and a small adrenal cortical tumor.

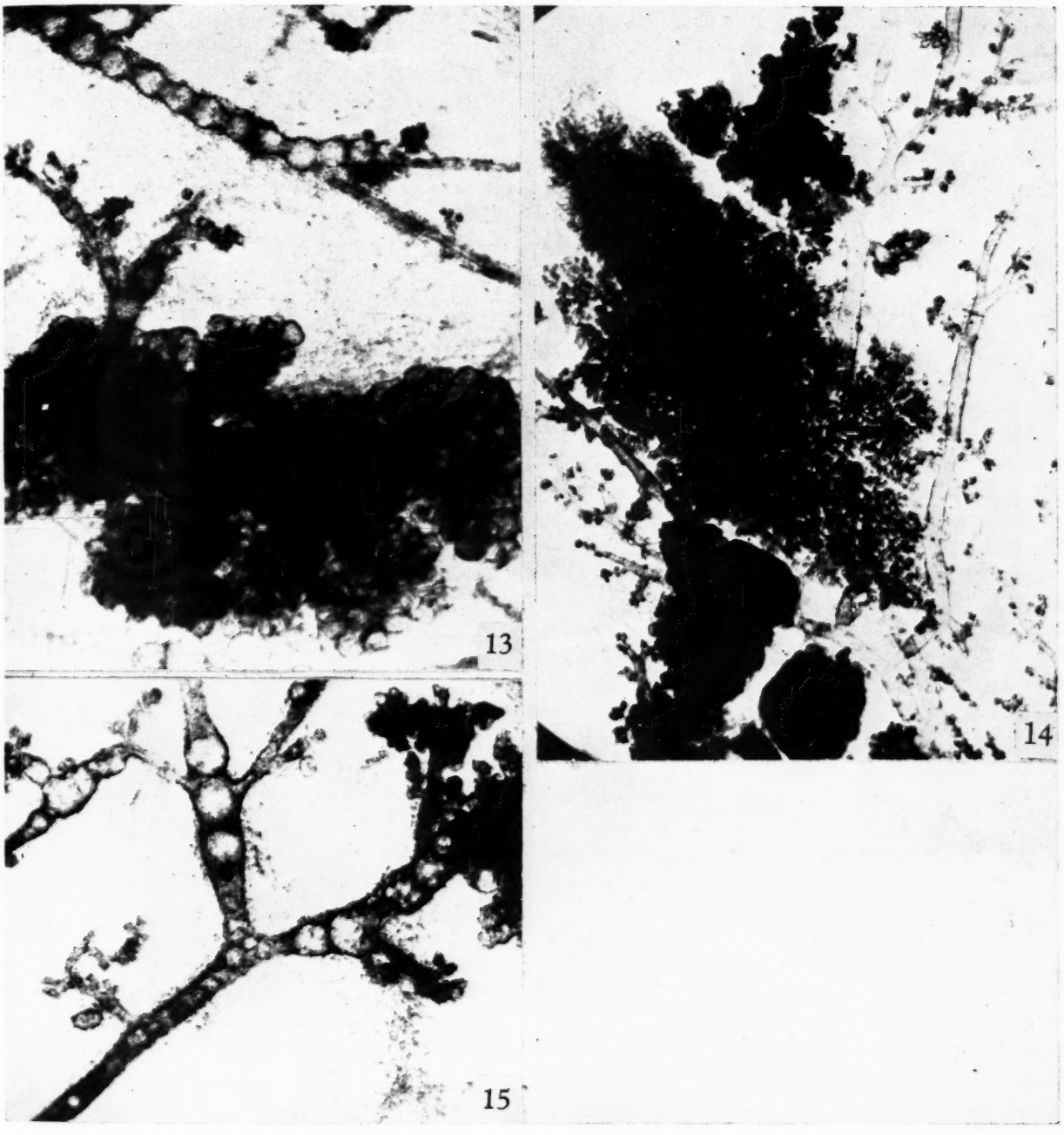
Photographs of one of the second pair of mammary glands from each of 4 mice of the CC_1 group which had received weekly injections of estradiol benzoate.

FIG. 9.—From a male mouse which had received 1.15 mgm., in 477 days, starting at 36 days. The gland consists largely of branching ducts and few small terminal ducts and alveoli. This mouse had a pituitary tumor weighing 96 mgm. and brown degeneration, Stage 4.

FIG. 10.—From a female which had received 1.166 mgm. in 492 days, starting at 33 days. A large number of small branches and alveoli were present. This mouse had a pituitary weighing 55.5 mgm., and brown degeneration, Stage 2.

FIG. 11.—From a female mouse which had received 1.316 mgm. in 556 days, starting at 37 days. Many small alveolar buds are present, increasing the parenchymal tissue. Nodules are lacking. This mouse had a pituitary tumor weighing 19 mgm., and brown degeneration, Stage 2, with early adenomatous proliferation of the adrenal cortex.

FIG. 12.—From a female mouse which had received 3.65 mgm. (50 μgm. weekly) in 513 days, starting at 47 days. The glands were very well developed and contained no nodules. This mouse had a hypophysis weighing 10 mgm., and brown degeneration, Stage 1.



Photomicrographs of areas of the whole mounts of mammary glands.

FIG. 13.—From a female mouse of the CC_2 group which had received 0.983 mgm. estradiol benzoate in 417 days, starting at 23 days. This mouse had a pituitary gland weighing 3.5 mgm., brown degeneration, Stage 2, and a large carcinoma of the uterine cervix. The mammary ducts contained a concretion-like material giving them a beaded appearance. The small nodule is composed of a mass of alveoli. Mag. × 90.

FIG. 14.—From the same gland shown in Fig. 5. The nodules and the diffuse adenomatous growth are shown at higher magnification.

FIG. 15.—From a female mouse of the CC_1 group which had received 1.133 mgm. estradiol benzoate in 481 days, starting at 36 days. This mouse had a pituitary gland weighing 12 mgm., and brown degeneration, Stage 2. The concretion-like secretory products distend the ducts. Mag. × 60.

among the hyperchromatic epithelial cells of these growths.

All of the above types of localized overgrowths were apparent in different mammary areas of individual mice of the CC_2 group. Only one nodule was found in the glands of the CC_1 group.

The incidence of mammary tumors in untreated mice of the parent strain and hybrids.—No mammary tumors developed in 17 female mice of the C57 strain. Seventy per cent or 12 of 17 female mice of the CBA strain had mammary cancers at ages ranging from 282 to 760 days. Two of the 3 females which gave birth to 78 of the mice of the CC_2 group died with mammary cancer at 329 and 609 days (Table III). The third died without a tumor at 450 days. The mammary glands of the mice of the CBA strain have been previously described although at that time the incidence of tumors was much lower (8).

Seventeen of 20 female mice of the CC_2 group died with mammary carcinomas (Table III, Fig. 22). The mammary carcinomas in the untreated mice of the CC_2 group were similar to those of the estrogen-treated mice. The general development of the mammary glands varied greatly as some were obtained from pregnant mice or at varying stages of regression. The number of glands with localized hyperplastic nodules was much smaller than in the treated group. The glands of several of the younger mice with mammary carcinoma contained no nodules. The number of nodules was greater in the older mice.

Mammary tumors were present at death in 43 per cent of the female CC_1 mice (Table III, Fig. 23). The mammary tumors in the untreated mice of the CC_1 group varied greatly in structure. Five mice repeatedly showed tumors attaining 1 to 2 cm. in diameter during two to five pregnancies. These tumors regressed following parturition. Three of these mice eventually died with mammary adenocarcinomas. Adenocarcinomas appeared in 5 CC_1 mice. The other tumors, frequently multiple, were composed of squamous cells. Several of these tumors were small, 4-6 mm. in diameter, and others were over 1 cm. The cords of squamous cells, some of which were large cyst-like structures filled with cornified cells, were surrounded by a dense stroma. In places the epithelial cells were pyknotic and regressing. Mitotic activity was usually limited to areas of smaller cords at the periphery. These tumors grew very slowly. Since they arose in very old mice they could not be observed for prolonged periods. Their progressive growth could not be determined. Histologically they cannot be considered to be rapidly growing and are probably not malignant. One tumor of a similar type, although more hyperplastic, appeared in one estrogen-treated CC_2 mouse.

TABLE III: THE INCIDENCE OF MAMMARY AND LYMPHOID TUMORS IN UNTREATED MICE OF THE TWO HYBRID GROUPS AND THE CBA STRAIN

Group	Sex	Number	Mammary tumors		Number lymphoid tumors
			Number	Per cent	
CC_2	F	20	17	85	1
	M	4	..	..	1
CC_1	F	30	13	43	2
	M	18	..	..	..
CBA	F	17	12	70	..
	M	9	..	..	..

Hyperplastic nodules were rarely found in the glands of the CC_1 mice. Several small nodules of cystic ducts or alveoli or cornified cysts were found. The latter could have been early tumors of the type mentioned above as occurring in these mice.

Adrenals.—The adrenal glands of the mice were studied from serial sections of their central portions containing the medulla. The size and histological picture of the glands varied extensively. The more obvious of these variations were the extent of brown degeneration and the disorganization of the cortex with or without the formation of adenomatous nodules. These morphological irregularities in the adrenal glands were then considered in relation to the condition of the mammary glands, sex, hybrid group, age, presence of mammary tumors and pituitary tumors.

DESCRIPTION OF FIGURES 16 TO 21

Photomicrographs of localized nodules and diffuse adenomatous nodules of estrogen-treated mice of the CC_2 group.

FIG. 16.—The greater part of one of the diffuse adenomatous nodules of a female mouse which had received 1.166 mgm. in 550 days, starting at 56 days.

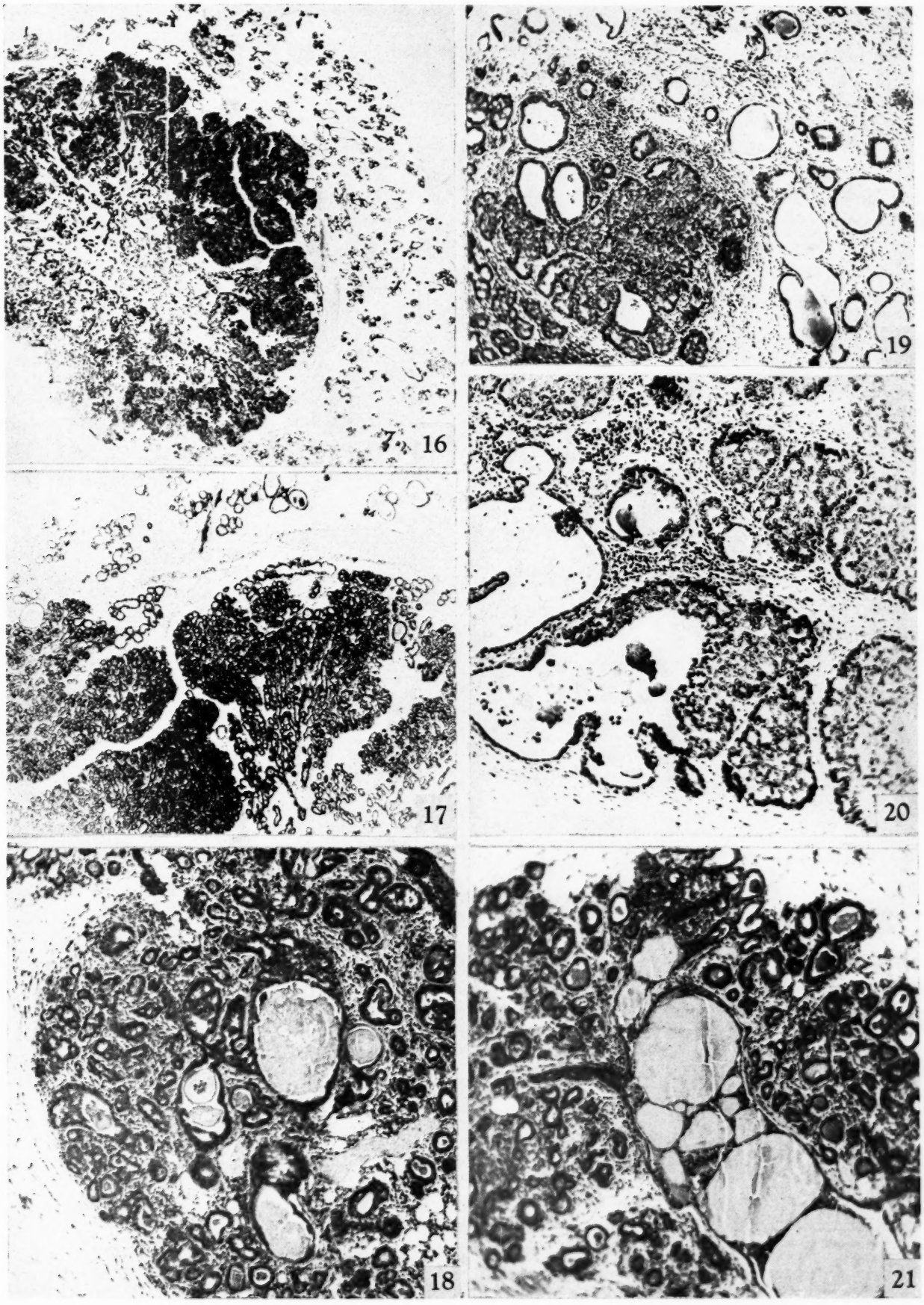
FIG. 17.—Higher magnification of the above.

FIG. 18.—A mammary nodule from a male mouse which had received 0.850 mgm. in 389 days, starting at 27 days. Several ducts contained accumulations of secretion. Some of the ducts and alveoli are lined by several layers of hyperchromatic cells. The stroma is dense and fibrous.

FIG. 19.—A portion of one of the nodules from a female mouse which had received 1.250 mgm. in 585 days, starting at 56 days. The hyperchromatic mammary epithelium of the nodule appears to be extending by progressive involvement of the surrounding tissue.

FIG. 20.—A portion at the periphery of a nodule showing the extension of the tumorous tissue through the surrounding tissue.

FIG. 21.—A second nodule from the mouse described above in Fig. 20. The large duct contained the concretion-like masses separated by epithelial cells.



FIGS. 16-21

The cortex of the adrenals of all except one of the estrogen-treated animals showed accumulations of a brown pigmented material (brown degeneration). The amount of cortical tissue involved varied from slight (Stage 1) to almost complete absence of the zona fasciculata (Stage 4). This particular type of degeneration apparently occurred first in the perimedullary region and progressed, with further accumulation of vacuoles and a brownish pigment, into the surrounding cortical cells which fused to form large multinucleate masses. In some adrenals the process also occurred in the outer layers of the fasciculata just beneath the glomerulosa. The degeneration either involved the cortex in a regular manner or irregularly with complete replacement of the fasciculata at some point or points and less involvement at other regions. The normal organization of some glands was so distorted that no definite layering of the cortex could be ascertained or the medullary border distinctly determined.

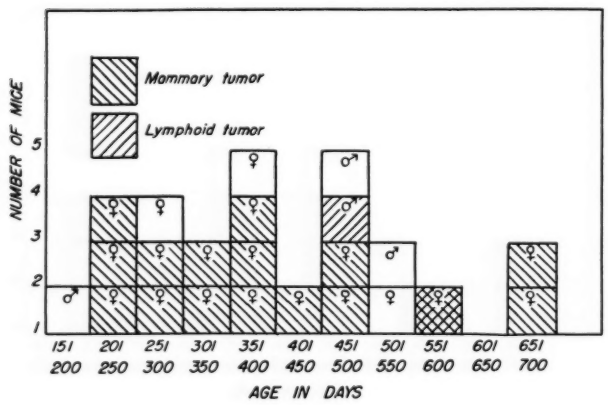


FIG. 22.—The age distribution and incidence of tumors in hybrid mice of the CC_2 group. The females were almost continuously pregnant.

The glomerulosa of many glands was either uniformly thickened or thickened at one or more areas. This condition usually was associated with accentuated degenerative changes in the deeper layers of the cortex. Many mitotic figures were found in the glomerulosa of some of the glands. Irregular projections of the small glomerulosa cells frequently extended into the lower layers of the cortices of some glands.

Small microscopic to larger grossly apparent adenomatous overgrowths of the adrenal cortices were occasionally observed. These overgrowths were distinct from the small capsular or pericapsular nodules of cortical tissue seen in the adrenal glands of many mice. The adenomatous growths showed extreme irregularity in the cell cords. Some of them contained large vacuolated and nonvacuolated cortical cells such as may be seen in the outer fascicular zone. Others contained, in addition, variable proportions of small glomerulosa-like cells. In some of the adrenal glands brown degeneration occurred in the adenomatous nodules.

The adrenal glands of the untreated mice were more uniform in size and structure. Brown degeneration infrequently appeared in more than traces (Stage 1) in the adrenals of mice of either group and was of similar incidence in mice of both groups. Likewise adenomatous hyperplasias of the adrenal cortices were very rare. A slight accumulation of amyloid or some similar material occupied the perimedullary region of the glands of some untreated mice of the CC_1 group. Amyloid also appeared in the livers and spleens of some of these mice.

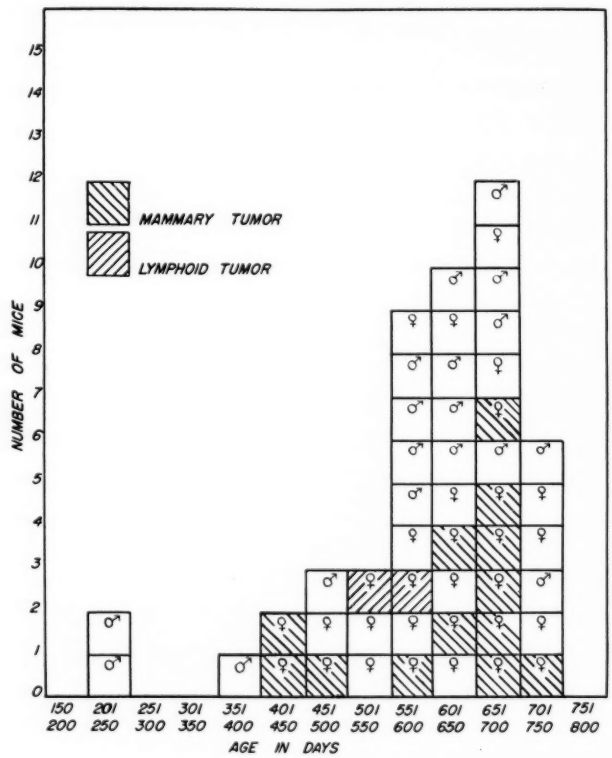


FIG. 23.—The age distribution and incidence of tumors in hybrid mice of the CC_1 group.

The most extensive degenerative changes occurred in the adrenals of the estrogen-treated males of both groups. The glands of most of the males showed stages of degeneration rated as 3 and 4, while most of the females fell in Stages 1 and 2 (Table IV). The amount of degeneration tended to be greater in the females treated for the longer periods and less extensive in those dying at earlier ages. The present material does not reveal a similar tendency in the males.

Since the incidence and extent of brown degeneration in the two groups of hybrids were similar no obvi-

ous relationship existed to the tendency to develop mammary tumors. The extent of adrenal involvement in the females of the CC_2 group was less than in the males, yet the incidences of tumors in the treated mice of both sexes were similar. The data in no way reveal any relationship between the extent or incidence of the adrenal degeneration and the tendency of either the estrogen-treated or control mice to develop mammary tumors.

As previously stated the incidence of hypophysial tumors and of advanced stages of brown degeneration of the adrenal glands were both greater in the males than in the females (Table V). This might indicate that the changes in these two glands are related. Such a tendency was indicated when weights of the hypophyses were plotted with the arbitrary rating of the extent of degeneration of the adrenal glands. Such criteria are crude, however, and the present data were only considered to indicate such a possibility.

TABLE IV: NUMBER OF HYBRID MICE AT DIFFERENT AGES SHOWING THE VARIOUS STAGES OF BROWN DEGENERATION OF THE ADRENAL GLAND

		CC_1 Stage of brown degeneration					CC_2 Stage of brown degeneration				
Age in days	Sex	0	1	2	3	4	0	1	2	3	4
100–200	M	..	..	..	..	..	..		..	..	..
	F	..	..	..	..	..	..	1	..	..	..
200–300	M	..	..	1	..	..	..	..	..	..	..
	F	..	..	2	1	..	1	2	1	..	..
300–400	M	..	..	..	..	..	..	1	..	2	..
	F	..	1	..	..	..	..	1	1	..	..
400–500	M	..	1	1	3	3	..	..	5	6	4
	F	..	2	..	1	..	..	2	..	..	..
500–600	M	..	..	3	..	4	..	..	3	1	2
	F	..	6	4	..	..	..	1	..	1	..
600–700	M	..	..	..	1	..	..	..	..	1	..
	F	..	..	1	2	..	..	1	1	..	..
Totals	M	..	1	5	4	7	..	1	8	10	6
	F	..	9	7	4	..	1	8	3	1	..

OTHER RESPONSES OF THE HYBRID MICE TO ESTROGENS

The seminal vesicles and prostates of male mice of the CBA strain receiving 16.6 μgm. of estradiol benzoate weekly rarely show the characteristic changes described and illustrated by Burrows and Kennaway (4). The accessory genital glands of males of the C57 strain almost always show such a response to continuous estrogenic treatment at this level. The mice of both groups of hybrids resembled the C57 strain in the responses of their accessory genital tissues. The coagulating glands were distended and greatly enlarged with accumulations of cornified cells from the metaplastic epithelium. The epithelium of the seminal vesicles underwent only a partial metaplasia of the proximal portions. The bladders of the hybrids were usually extremely distended and hydronephrosis appeared frequently. No significant variation in the response of the accessory genital tissues was evident between the males of the two groups.

Testes.—The testes were rather uniformly reduced in size. The seminiferous epithelium revealed variable extents of degeneration, although primary spermatocytes were found in most of the testes. The interstitial tissue usually contained large multinuclear cells which resembled the brownish masses in the adrenal glands. Slight hypertrophy or hyperplasia of the glandular interstitial cells appeared in some of the testes but the lesions were small.

Skeletal tissues.—The femurs of all of the mice were examined. Many of them were broken in order to determine their breaking strength and also sectioned and photographed by x-rays (13). All the mice showed the usual increased deposition of bone in the medullary cavities as has been previously described and the pubes were partially reabsorbed at the symphysis.

Connective tissue.—A spindle celled sarcoma appeared in the subcutaneous tissue of one male mouse of the CC_2 group and in one mouse of the CC_1 group, in relation to the sites of injections of the oily solution of estrogen.

Lymphatic system and blood.—Lymphoid tumors appeared in 7 mice of the CC_2 group and in 5 mice of the CC_1 group (Table II). The mediastinum was extensively involved in all of the mice. The tumors were usually detected because of the respiratory difficulty. The mediastinal tumors invaded the intercostal muscles, lungs, pericardium, and occasionally the heart. In 5 mice a generalized leukemic infiltration of the liver, kidney, genital tumors, and all adenoid tissues

occurred. Extramedullary hepatic hemopoiesis was commonly seen in the hybrid mice, both untreated and treated. In this respect they resembled again the C57 parent. Four of the mice of the control group also died with lymphoid tumors.

Discussion

Neoplasia of the mammary and pituitary glands in mice has been associated with the administration of estrogen. It has been demonstrated that the realization of the tumors of both of these glands is also dependent upon inherent, presumably genetic, factors in mice of different strains. Another influence transmitted from mother to offspring, apparently through the milk, also determined the incidence of breast tumors in estrogen-treated mice (24).

The present experiments on the first generation of hybrid mice obtained by mating animals of a strain showing susceptibility to mammary cancer with animals presenting a strain-limited susceptibility to hypophysial tumors demonstrated the maternal influence on the appearance of mammary neoplasia. The above observations permit an interpretation of an extra chromosomal factor in the transmission of mammary cancer (22) and are consistent with the existence of an active influence transmitted by the milk from cancer-susceptible mother to the suckling young (2, 24). The females of the CBA strain transmitted to their hybrid offspring a tendency to develop mammary tumors materializing in mammary neoplasia in the estrogen-treated males and females as well as in the breeding females. This tendency was not transmitted by males of the CBA strain to their hybrid offspring to a comparable extent since none of the estrogen-treated animals and a much lower percentage of the breeding females acquired mammary tumors.

Tumors of the hypophyses appeared in mice of the C57 strain which had received estrogens (12). The tendency to give rise to such tumors was transmitted by both the males and females of the C57 strain to their first generation hybrid offspring which received estradiol benzoate. The hybrid mice with the C57 mother showed a slightly higher incidence of hypophysial tumors but the difference cannot be considered significant at this time. The transmission of the tendency for estrogen-treated mice to develop hypophysial tumors is compatible with a genetic interpretation.

Not only the tendency to develop mammary cancers but also the tendency for the formation of non-malignant or premalignant overgrowths varied greatly in structure as previously mentioned. (10). The mammary glands of mice of strains susceptible to mammary tumors have uniformly shown these localized

Table V: The Size of Pituitary Tumors and the Extent of Adrenal Degeneration in Hybrid Mice Receiving Estrogens *

Wt. of pituitary in mgm.	Sex	BD-0 †		BD-1 †		BD-2 †		BD-3 †		BD-4 †	
		CC_2	CC_1	CC_2	CC_1	CC_2	CC_1	CC_2	CC_1	CC_2	CC_1
0–10	M	..	..	1	..	..	..	1	1	..	..
	F	1	..	6	5	4	3	1	1	..	..
11–20	M	..	..	..	..	1	1	5	..	..	..
	F	..	..	1	2	3	3	..	..	..	..
21–30	M	..	..	..	..	..	..	..	2	1	2
	F	..	..	..	1	..	..	..	..	..	..
31–40	M	..	..	..	..	..	..	..	..	3	..
	F	..	..	..	1	2	1	..	1	..	..
41–50	M	..	..	..	..	..	1	2	..	..	1
	F	..	..	1	..	..	..	..	..	..	..
51–60	M	..	..	..	..	..	..	..	1	..	1
	F	..	..	..	..	..	1	..	..	..	..
61–70	M	..	..	..	..	..	1	..	..	1	..
	F	..	..	..	..	..	..	..	..	..	..
71–80	M	..	..	..	..	..	1	..	..	..	..
	F	..	..	..	..	1	..	..	1	..	..
81–90	M	..	..	..	..	..	..	2	..	1	1
	F	..	..	..	..	..	..	..	..	..	..
91–100	M	..	..	..	..	..	..	..	..	..	1
	F	..	..	..	..	..	..	..	..	..	..
101+	M	..	..	1	1	..	..	1	..	1	
	F	..	..	..	..	..	..	..	..	..	..

* Neither adrenals nor pituitary glands of all the mice were saved, hence the above table does not include all of the animals.
† BD = brown degeneration. Numbers denote stages.

overgrowths (10, 25). The number of nodules in the glands of the different tumor-susceptible strains is not, however, necessarily proportional to the incidence of mammary cancer attained. The injection of estrogen increased the number of these nodules in mice of the CC_2 group but not the incidence of mammary cancer when compared with the breeding females. It is, therefore, possible that estrogenic treatment directly or indirectly was responsible for their frequent development. Since the incidence of carcinomas was not greater in the estrogen-treated mice, although the number of nodules was much greater, a relation between the localized overgrowths and malignant growths might be questioned. On the other hand the presence of histologically malignant areas in some of the nodules indicated a relationship to malignant degeneration. The rather frequent regressive changes seen in many of the nodules indicates that they are often incapable of progressive phases of atypical growth. Some of them may proceed through these reversible stages and become irreversible. The histological stages at which the atypical localized nodules develop a complete autonomy is not known. The injection of estrogen in tumor-susceptible animals is followed by the appearance of many localized atypical, nonmalignant hyperplasias. The present experiments demonstrate that the proper maternal or milk factor is also necessary for this development.

The pituitary glands of mice of the mammary tumor-susceptible RIII strain hypertrophied when estrogens were injected (5, 6, 17). On the other hand, in this laboratory the low-tumor C57 strain was the only one of the nine studied in which adenomatous hypertrophies and adenomas of the hypophyses appeared frequently during estrogenic treatment. These observations would indicate that a relationship did not exist between the hypophysial and mammary responses. In the present experiments genetically similar animals, one group of which was highly susceptible to mammary tumors and the other not, both developed pituitary tumors. Pituitary tumors or hyperplasias and mammary cancers have been reported in one untreated mouse (8), and in untreated rabbits (14, 15) and rats (26) of certain strains. If one might generalize from these observations the above hypophysial-mammary relationship might be considered to result from a common etiological factor rather than to be interdependent.

The histopathological states of the adrenal glands of neither the treated nor control groups could be related to the tendency to develop mammary tumors. It is possible that the condition of the hypophysis might be related to the degenerative changes of the adrenal cortex since the male mice of both groups showed the most extensive lesions of both glands. The tendency of the extent of brown degeneration of the adrenal glands could not be correlated with susceptibility for spontaneous mammary cancer in mice in this laboratory (3).

The response of the accessory genital organs of the males, the marked tendency for urethral obstruction and urine retention and the appearance of hepatic hemopoiesis which appeared in estrogen-treated mice of the C57 strain also occurred frequently in the hybrid mice of both groups.

The increased incidence of malignant lymphoid tumors in estrogen-treated mice of both groups is entirely compatible with previously reported experiments on mice of other strains (11). The amount of estrogen used in the hybrid mice resulted in a comparable increased incidence of lymphoid tumors in mice of the C3H strain and larger doses in a higher incidence. The incidence of lymphoid tumors in the untreated hybrids was higher than in untreated mice of either of the parent stocks.

Summary

1. Mice of a mammary tumor-susceptible strain (CBA) were mated to mice of a mammary tumor-resistant strain (C57). Seventy per cent of the females of the former strain and none of the females of the latter strain developed mammary adenocarcinomas.

2. Fifty-two hybrid mice with CBA mothers and 53 hybrids with C57 mothers received injections of 16.6 or 50 μgm. of estradiol benzoate weekly throughout life. Other mice of similar origin were untreated but permitted to breed, the young being removed at birth.

3. Mammary cancers appeared in 59 per cent of the estrogen-treated male and female mice with CBA mothers and in 85 per cent of the breeding hybrid females of similar origin. None of the estrogen-treated hybrid mice with C57 mothers developed mammary tumors, although 43 per cent of the breeding females had mammary tumors at death. Only about one-third of the tumors in the latter group were considered malignant. The environment of mammary glands in females subjected to continuously repeated pregnancies predisposed to a higher incidence of mammary tumors than the environment created by administration of estrogen.

4. Nonmalignant and premalignant localized mammary growths appeared in the estrogen-treated hybrid mice with the tumor-susceptible CBA maternal parents, but were lacking in the mice with C57 mothers. The tendency for localized overgrowths was acquired with the tendency to develop a high incidence of mammary tumors.

5. Hypophysial tumors, which had appeared previously only in estrogen-treated mice of the C57 strain in the laboratory, appeared in the estrogen-treated hy-

brids of both groups. The incidence was greater in the males than in the females. The transmission of the tendency to develop such tumors is compatible with a genetic interpretation.

6. The incidence of lymphoid tumors was greater in the estrogen treated hybrids than in the controls.

7. The adrenal glands of the mice revealed no histological changes that could be associated with the tendency to develop mammary tumors.

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Cancer of the Cervix of the Uterus in Hybrid Mice Following Long-Continued Administration of Estrogen*†

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The growth stimulating action of estrogen on the female genital tissues first focused attention upon the possibility of inducing cancer of the cervix of the uterus by long-continued administration of this hormone (2).

These experiments were first undertaken in the monkey. Atypical hyperplastic lesions were obtained which were considered "precancerous" (26, 27). Continued experiments in monkeys have so far failed to induce definite cervical cancer (3).

In guinea pigs long-continued injections of estrogen, although producing fibromyomas and glandular cystic hyperplasias of the uterus (25), have failed to produce cervical cancer.

Carcinoma of the uterine cervix does not appear spontaneously in mice. In this respect it differs from mammary cancer. Unlike mammary cancer (31), it has not been induced by local application of carcinogens. After long-continued treatment with estrogen, however, cervical lesions in mice have been reported by several investigators (10, 13, 14, 24, 28, 29, 32). In these earlier reports the incidence was less than 15 per cent of the treated animals which lived beyond certain definite ages (usually a year or 15 months).

One reason why the incidence of cancer of the cervix uteri has been so low in mice treated with estrogens is that mammary cancer appears at an earlier age than does cervical cancer, and consequently animals may die of the former; *i.e.*, many of them do not live long enough for the cervical cancer to develop. As previously reported (14), it was necessary to remove mammary tumors twice before the appearance of an advanced, transplantable carcinoma of the uterine cervix.

In our experience other mice from strains less susceptible to mammary tumors have not tolerated prolonged treatment with estrogens at high levels (13).

In mammary cancer in mice (a) hereditary constitution (22) (b) estrogenic stimulation (21, 23), and (c) maternal transmission (milk factor) (8), are all important factors in carcinogenesis. As female mice age and the mammary glands atrophy, multiple hyperplastic nodules are present (19) which provide several potential loci of origin of mammary cancer. The milk factor plays an important part in determining the presence of these hyperplastic nodules (11). Ovarian hormones or estrogenic stimulation are necessary for the growth of the tissues of the mammary glands. (1, 15, 20, 23, 33).

The present paper reports an incidence of cervical lesions and carcinoma of 50 per cent in one group of 20 mice, and an incidence of 62 per cent in a second group of 24 mice, which survived estrogenic treatment for more than one year. Previous work has been limited to inbred strains. This report involves hybrids between two strains inbred in regard to mammary cancer.

This high incidence focuses attention on estrogenic hormone as a very important, rather than an incidental factor in cancer of the uterine cervix.

Animals and Treatment

Two strains of mice were chosen, one of which, C57, is definitely resistant to mammary cancer. The other, CBA, has an incidence of about 70 per cent of spontaneous mammary tumors in "forced bred" animals, but these tumors appear relatively late in life. As previously reported, C57 mice have a high incidence of pituitary tumors (18). C57 animals have poor tolerance for estrogenic treatment, while CBA mice tolerate this treatment well. Therefore these two strains were hybridized in the hope that the F1 generation would tolerate estrogenic treatment well and that the incidence of hypophysial and mammary tumors might be determined.

Table 1: Qualities of CBA and C57 Mice

Parent strain	Incidence of: Spontaneous mammary cancer	Incidence of: Pituitary tumors when on estrogenic treatment	Tolerance of estrogenic treatment
CBA	Medium (70%)	None (0%)	Good
C57	Resistant (0%)	High (40 + %)	Poor

In order to study possible influence of maternal transmission (milk factor) two crosses were made as follows:

1. *Between C57 females × CBA males:* This group was designated as CC_1, and 53 of the F1 generation animals of both sexes were injected. Twenty-four females survived estrogenic treatment for more than one year.

2. *Between CBA females × C57 males:* This consti-

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tuted group CC_2, and 52 of the F1 generation of these hybrid animals were placed on experiment. Six of these mice died before one year of age, 5 of them of mammary cancer. Twenty females survived estrogenic treatment for more than one year.

Cancer of the cervix was not found in animals of either group sacrificed before one year of age. One large, transplantable carcinoma of the cervix of the uterus in a mouse 364 days of age had been reported previously (14).

Previous experiments with estrogenic induction of mammary tumors (17) indicated the probable optimum dose at between 15 and 25 μgm. In this experiment 16.6 μgm. per week was used in the majority of animals. Some received as much as 50 μgm. per week. Estradiol benzoate was the estrogen. Other cervical cancers have been obtained after injecting other estrogens; stilbestrol, triphenylethylene, estrone, and estradiol dipropionate, and also after combinations of estrogens with androgens and progesterone (12).

Treatment was begun at 28 to 56 days of age and continued at the same level throughout the animals' lives.

Similar numbers of untreated hybrid mice were maintained as controls. Since it had been shown that pregnancy definitely increased the incidence of mammary cancer, the controls were mated and the young removed immediately after birth. Since female mice ovulate the day after parturition, and males were always present, these control females were almost continually pregnant (7), at least during their early reproductive life.

The mice were all from Dr. L. C. Strong's colony, kept in air-conditioned environment and fed on Purina Fox Chow. It has previously been shown that mice kept on a diet of Fox Chow have a higher incidence of mammary cancer than those kept on one other diet (30). No data are available for estimating the possible effect of diet on cervical cancer.

Results

The cervical cancers here reported are squamous cell carcinomas. They originated in the stratified epithelium of the lower cervical canal, external cervical os, or the fornices of the upper vagina. The most common site was the posterior lip of the cervix with its frenulum-like fold to the posterior wall of the vagina. The parts of the cervix and vagina involved by the lesion are shown in Fig. 1 for four of these tumors.

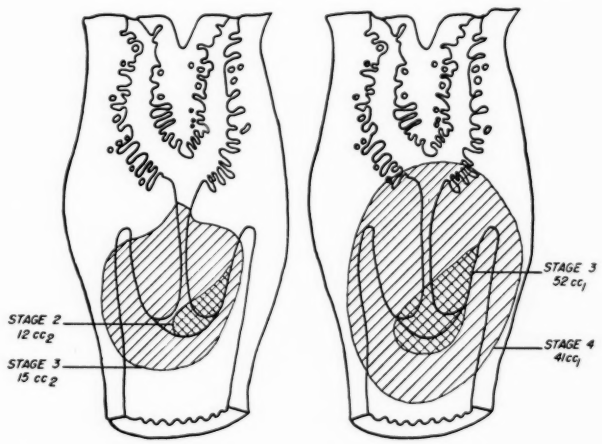


Fig. 1.—Diagrams of coronal sections through the cervix uteri and upper vagina showing the dorsal half to indicate the extent of 4 of the lesions in animals 12 CC_2, 15 CC_2, 52 CC_1, and 41 CC_1 (see Figs. 9, 10).

For purposes of classification the lesions have been divided into 4 stages (13), all of which are considered definitely pathological, as judged by histological criteria. Stages I and II are early invasive lesions, usually localized and limited to the mucosa, the posterior lip and external os being the most frequent locations. These lesions are frequently quite hyperplastic and may contain epithelial pearls (Figs. 1 and 2). Stage IV is an extreme lesion involving practically the whole cervical canal and the upper part of the vagina,

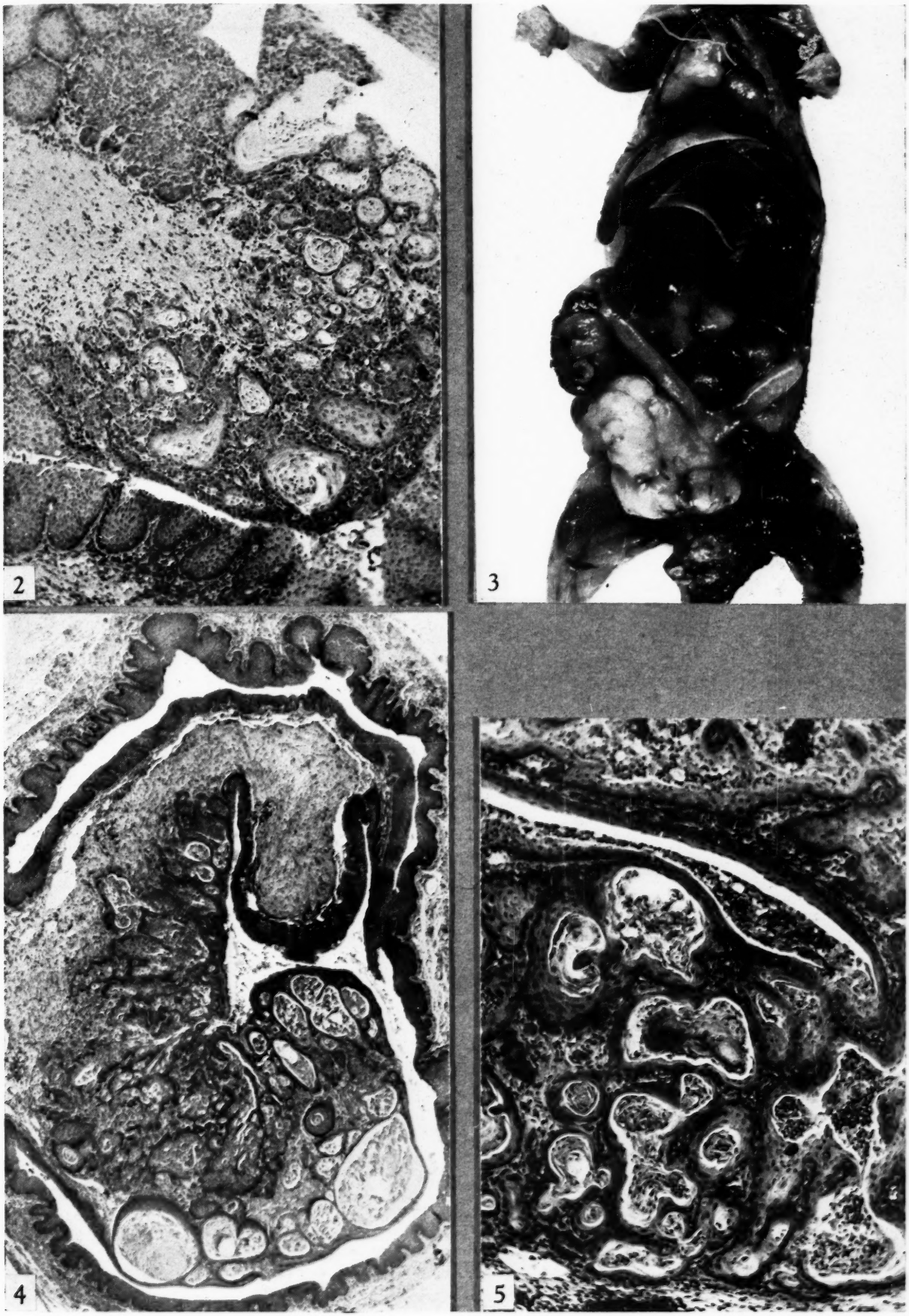
DESCRIPTION OF FIGURES 2 TO 5

Fig. 2.—Stage II lesion in mouse 12 CC_2, involving the posterior lip and the left side of the external os of the cervix, but not the adjacent vagina (see Fig. 1–left for the extent of the lesion). The mouse was 508 days old and had received 1.33 mgm. estradiol benzoate in 68 weeks, starting at 27 days of life. She also had a mammary adenocarcinoma in the 4th left gland and a pituitary tumor weighing 20.5 mgm.

Fig. 3.—Stage IV lesion in mouse 11 CC_2. Photograph of a cervical carcinoma in a mouse treated for 528 days, total dose 1.25 mgm. estradiol benzoate. Besides this Stage IV squamous cell carcinoma, this mouse also had lymphatic leukemia.

Fig. 4.—Stage III lesion in mouse 52 CC_1 involving the posterior lip and the left side of the cervix and also the posterior wall of the upper vagina (See Fig. 1–right). The section is through a lower level at the opening of the cervical canal. The frenulum-like fold connecting the posterior lip of the cervix with the posterior vagina is shown in the left center. This cervix was greatly enlarged, fibrous, and nodular. This mouse had a large tumor in the right ovary, and a pituitary gland weighing 10 mgm. She had received 3.65 mgm. estradiol benzoate in 73 weeks.

Fig. 5.—Stage IV lesion in mouse 15 CC_2. A squamous cell carcinoma involving the entire cervix and upper vaginal wall. The cervix was enlarged, fibrous, and nodular. This mouse had received 1.2 mgm. estradiol benzoate in 72 weeks; had also a mammary adenocarcinoma in the left 5th gland and a pituitary weighing 7 mgm. (See Fig. 1–left.)



FIGS. 2-5

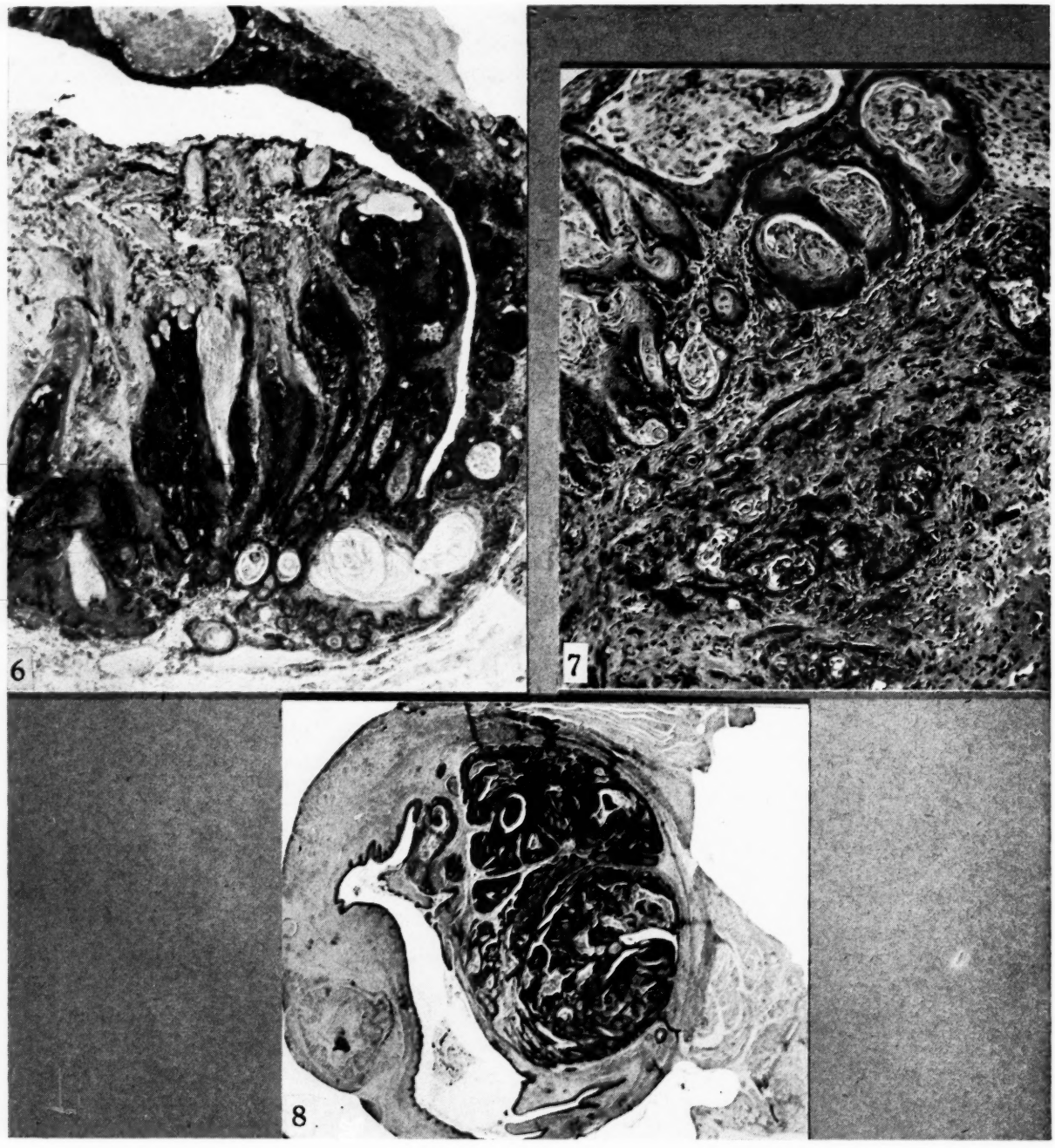


FIG. 6.—Stage IV lesion in mouse 53 CC_1. This tumor involved the whole right side, posterior lip, and part of the left side of the cervix and extended well down into the vagina. It was observed in the living animal and watched for several days before autopsy. The surface protruded into the vaginal canal and was necrotic. This animal had received 3.7 mgm. estradiol benzoate in 74 weeks, and had a pituitary tumor weighing 28 mgm.

FIG. 7.—Stage IV lesion in mouse 41 CC_1. A squamous cell carcinoma involving the entire cervix from the bifurcation of the uterine horns and including the upper portion of the vagina. The invasive nature of the small cords of epithelial cells is shown. The tumor was discovered at autopsy but not observed in the living animal. Fig. 8—right shows the extent of the lesion. This mouse had received 1.35 mgm. estradiol benzoate in 81 weeks. The animal had a pituitary tumor weighing 89 mgm.

FIG. 8.—Stage IV lesion in mouse 3 CC_1. This lesion was a squamous cell carcinoma observed in the living animal and followed for several days before autopsy. The tumor was over 2 cm. in diameter and involved the entire vaginal and cervical regions. The figure illustrates the invasion of the tumor at the lower (vaginal) border. This mouse had received 1.116 mgm. estradiol benzoate in 67 weeks, and had a pituitary weighing 8.5 mgm.

showing extensive areas of rapid hyperplasia and penetrating to the walls of the rectum or the bladder. These tumors may be 2 cm. or more in diameter. A few may grow so extensively as to fill the whole pelvis (Figs. 3, 6, 7, 8), or to protrude from the vagina. Stages II and III are intermediate (Figs. 2, 4).

Suntzeff, Burns, Moskop, and Loeb (11) have also made a subdivision into 4 stages. It seems to us that their stages 1 and 2 were earlier developments than our Stage I, and sometimes on the borderline of normal. We have purposely not included this type as a lesion. Our Stage I definitely includes lesions recognized as early invasive growths. Since they are localized to the genital tissues, and since their progressive growth cannot be followed, we do not consider them as necessarily malignant. The sequence of transitional changes from the earlier lesions to the malignant ones have indicated that they are probably early stages of the same process.

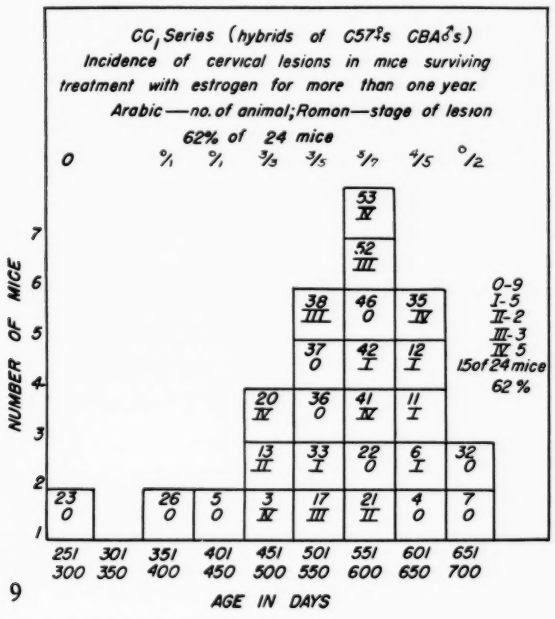
The incidence of lesions in these stages in the two groups of mice is indicated in Table II.

The age distribution is shown on the abscissa in Figs. 9 and 10. The various stages of atypical growth are designated by the Roman numerals. Each Arabic number designates the animal in which the lesion occurred.

The highest incidence of cervical lesions appeared in the CC_1 group, in which no mammary tumors developed. The lower incidence in the CC_2 group was probably due to the 60 per cent incidence of mammary tumors which occur at an earlier age than cervical lesions.

Inevitably the question arises as to the malignancy of cervical lesions in these experimental animals. For instance, as late as 1938 Suntzeff, Burns, Moskop, and Loeb (32) still preferred to designate these lesions as "cancer-like" because of the difficulty of proof of malignancy. However, the report in 1938 of continued growth of cervical cancer when transplanted to other mice of the same strain, without continuance of estrogenic treatment of the hosts, demonstrated the autonomy of this cancer growth (14). This tumor also metastasized to the regional lymph nodes, thus providing very good evidence of the malignancy of induced cervical carcinoma. In the present series no metastases were noted but at least 3 cancers were lethal. Also those rated as Stage IV showed direct invasion of adjacent nongenital tissues.

The estrogen-treated CC_1 animals (nursed by mothers of the C57 cancer resistant strain) had no mammary cancers, while the CC_2 animals (nursed by the CBA mothers, of a cancer-susceptible strain) had a 62 per cent incidence of mammary cancer. This stresses


CC1 Series (hybrids of C57♀s CBA♂s)
Incidence of cervical lesions in mice surviving treatment with estrogen for more than one year.
Arabic—no. of animal; Roman—stage of lesion
62% of 24 mice
0 0/1 0/1 3/3 3/5 5/7 4/5 0/2
NUMBER OF MICE
0-9
I-5
II-2
III-3
IV 5
15 of 24 mice
62%
251 300 301 350 351 400 401 450 451 500 501 550 551 600 601 650 651 700
AGE IN DAYS
9

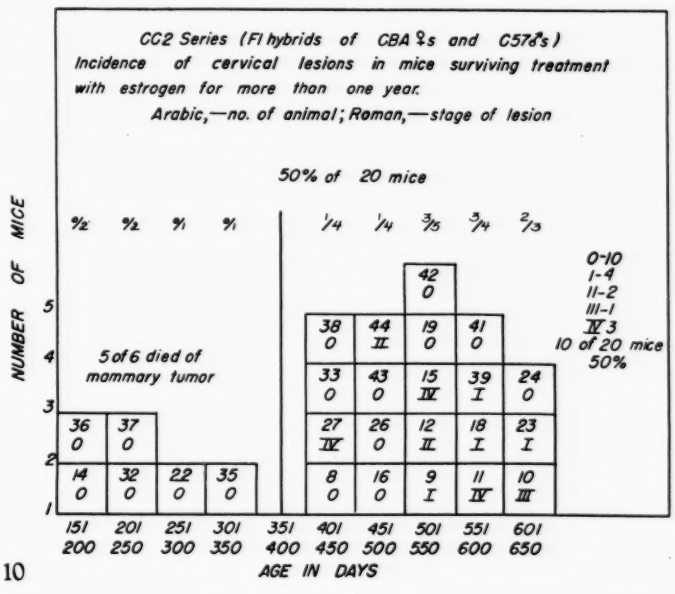

CC2 Series (F1 hybrids of CBA ♀s and C57♂s)
Incidence of cervical lesions in mice surviving treatment with estrogen for more than one year.
Arabic,—no. of animal; Roman,—stage of lesion
50% of 20 mice
0/2 0/2 0/1 0/1 1/4 1/4 3/5 3/4 2/3
NUMBER OF MICE
5 of 6 died of mammary tumor
0-10
I-4
II-2
III-1
IV 3
10 of 20 mice
50%
151 200 201 250 251 300 301 350 351 400 401 450 451 500 501 550 551 600 601 650
AGE IN DAYS
10

FIG. 9.—CC_1 series. Incidence of cervical lesions in mice surviving treatment with estrogen for more than one year.
FIG. 10.—CC_2 series. Incidence of cervical lesions in mice surviving treatment with estrogen for more than one year.

TABLE II: INCIDENCE OF CERVICAL LESIONS, LISTED IN 4 STAGES

Series of animals	Stage of lesion I	II	III	IV	Total lesions	Total animals	Incidence cervical cancer or invasive lesions
CC_1*	5	2	3	5	15	24	62+%
CC_2†	4	2	1	3	10	20‡	50%

* F1 hybrids of C57 females × CBA males.
† F1 hybrids of CBA females × C57 males.
‡ Five of 6 other CC_2 hybrids died of mammary tumors before 350 days of age.

maternal transmission as a factor in mammary carcinogenesis (8). The lower incidence of cervical cancer in the CC_2 hybrids is probably due to the early death of these mice with mammary tumors.

Maternal transmission (milk factor) does not seem to affect the incidence of carcinoma of the uterine cervix.

No cervical lesions appeared in either group before 400 days of age. None appeared in the CC_1 group before 450 days of age (Figs. 9, 10). The conclusion is therefore drawn that with this hybrid stock chronic estrogenic stimulation at this level usually requires longer than 400 days for the appearance of cervical cancer. Some of the animals lived 650 or 700 days, nearly 2 years, without developing cervical cancer (Figs. 9, 10, Stage 0).

As previously noted (13), cervical cancer seems to develop in all strains of mice when treated with estrogens, regardless of their resistance or susceptibility to mammary cancer, if they will tolerate the estrogenic treatment.

In the animals under treatment with estrogen which did not develop cervical cancer the proliferation of vaginal epithelium usually continued, in many cases to a condition equivalent to that of estrus, even in very old animals. Other signs of atypical changes occurred in both vagina and uterus of these animals and also in those in which cervical carcinoma appeared (13, 32). The most striking of these changes was the hyaline transformation of the connective tissues. The walls of blood vessels of the uterus, cervix, and vagina, especially the small arteries, showed striking hyaline transformation, almost to the point of closure of the lumen in some cases. There was also extreme growth of the glands of the uterus proper. Sometimes they grew through the muscle layers and the tips appeared as distended vesicles under the peritoneum. Less frequent, but present in several animals, was metaplasia of the uterine glands and surface epithelium. This was usually associated with evidence of pyometra.

One of the most interesting findings in the old mice which survived prolonged treatment without cervical lesions, was the unevenness of growth of the vaginal epithelium. Patches of thick stratified and cornified epithelium were frequently interspersed with other thin areas devoid of cornification and sometimes infiltrated with leukocytes.

In many of the cervical lesions it was relatively easy to find penetrating down-growths of epithelium with high mitotic indices indicative of extreme hyperplasia. In cases where extreme cornification of the lesions occurred, intermediate stages showed primarily a down-growth of epithelial bulbs into the connective tissue, and secondarily, the continued proliferation of this epithelium leading to production of a central core of cornified material. Later continued growth often produced an extreme cornified core and frequently leukocytic infiltrations appeared here, just as they appear periodically after estrus in the normal vagina of rodents.

Frequently there were areas of almost complete erosion of the epithelium. Usually these contained some leukocytic or round cell infiltration. Frequently a definite zone of round cells separated the thickened basement membrane from the hyalinized connective tissue.

Discussion

The growth of epithelium of the vagina and cervix of the uterus is entirely dependent upon hormonal factors in normal life. Therefore, knowledge of the fluctuation of the structure of these organs in normal rodents is necessary for an appreciation of the influence of estrogen in abnormal growths.

The structure of the vagina in the normal animal seems entirely dependent upon the level of estrogenic hormone (6). Before puberty the vaginal wall is composed of only 2 layers of epithelial cells (5). During the first estrus, which leads to puberty, the vaginal wall grows rapidly so that in the course of 2 or 3 days it becomes a thick stratified epithelium with a superficial cornified layer. After estrus passes, the superficial part of the wall is sloughed into the lumen and destroyed by leukocytic invasion. This leaves an epithelium between estrus periods of 5 or 6 layers in thickness.

A low stratified epithelium 2 to 4 layers of cells in thickness, extends through the external os well into the cervical canal to the point where the canal branches to enter the 2 uterine cornea. This is the point of transition between low stratified, cervical, and the columnar uterine epithelium.

As previously noted, the cervix of the mouse is different from that of women and monkeys in that it contains no mucous secreting cervical glands.

The most vulnerable part of the cervix is the posterior lip where the frenulum-like fold makes an attachment to the posterior vaginal wall. The early stages of cervical lesions which can be definitely localized occur most frequently here. Later stages extend both upward into the cervical canal and into the vaginal fornices as the tumors progressively invade extensive areas.

After the animal becomes pregnant, estrus cycles are postponed and the epithelium of the vagina, instead of becoming cornified as at estrus, develops a superficial stratum of mucous cells (6). This structure persists throughout pregnancy.

When the ovaries are removed from adults, the vagina returns to a 2- or 3-layered condition, indicating extreme atrophy. This is the condition typical of old age. Also younger animals in which spontaneous mammary cancers develop show precocious atrophic vaginal changes associated with ovarian atrophy (4).

Perry (28) and Perry and Ginzton (29) reported 3 cases of cervical carcinoma in a group of 27 mice (11 per cent) which had been painted on the back with estrone and dibenzanthracene and which survived more than 6 months of this treatment.

In 1938, Suntzeff, Burns, Moskop, and Loeb (32) reported an incidence of 10.7 per cent; 25 mice out of a total of 234 showed invasive or "cancer-like" lesions.

Gardner and Allen (13) reported 27 cervical lesions in 183 mice, an incidence of 14.2 per cent. Also Gardner, Allen, Smith, and Strong (14) reported the transplantability of cervical carcinoma through 3 generations of transplants, indicating that this type of growth is malignant.

In comparing these animals treated for long periods of time, often extending through the second year of life, it is clear that estrogenic treatment at this level maintains extensive growth of the uterus and vagina beyond the time at which this usually declines, and often practically stops; *i.e.*, the time equivalent to the menopause in women. Frequently, however, in old animals this growth may be uneven or spotty.

Other conditions present in these animals indicate the successful maintenance of a high level of estrogen. The symphysis pubis was resorbed and replaced by long pubic ligaments (9), and new osseous tissue had formed in the marrow cavities of the long bones (16).

Cervical carcinomas or invasive lesions appeared in the hybrid animals described above only after at least one year's treatment. Since the smaller lesions cannot be diagnosed until after death, there is no way of telling how long before autopsy they might have been present.

As has been reported (14), in one estrogen-treated mouse of the C3H strain, however, a malignant (transplantable, metastasizing) carcinoma occurred. This mouse at 45 days of age (about the time of puberty) was treated with estradiol benzoate, 16.6 μgm. being given every 3 weeks until the age of 266 days, by which time a total of 183 μgm. had been injected. Toward the end of this period a mammary tumor appeared and this was removed at 266 days of age. At this time the dose was tripled to 16.6 μgm. weekly. At 290 days of age a secondary mammary tumor was removed. At 359 days of age enlargement of the pelvis was noted and at 364 days a tumor protruded from the vagina. The mouse was sacrificed at 364 days of age, one day short of a year.

The tumor proved to be a large cervical carcinoma extending up to the bifurcation of the uterus. It surrounded the rectum, filled the pelvis, and infiltrated the muscles of the bladder. It was an extremely hyperplastic squamous cell carcinoma. The lumbar lymph nodes were enlarged, and hyperplastic metastases were identified in sections.

Deep portions of the carcinoma were implanted into 1 female and 4 males of the same strain. No hormone treatment was given to these hosts. All grafts grew to 1 cm. in diameter in 11 days. Three had infiltrated the skin by the 17th day. This cancer was carried through 3 transplant generations, in males as well as females, thus contributing evidence as to its autonomous growth potentialities.

Summary and Conclusions

After long treatment with high doses of estrogen, lesions and carcinomas of the uterine cervix have appeared in 15 of 24 mice (62 per cent) and 10 of 20 mice (50 per cent) which survived the treatment for more than one year. These two groups were hybrids of the C57 and CBA strains, inbred with regard to mammary cancer.

Genetic factors involved in mammary cancer seem to have little influence as determining factors in cancer of the cervix uteri.

The two groups mentioned above were formed to test the possible importance of maternal transmission (milk factor) in cervical cancer. Although important in mammary cancer, maternal transmission seemed to have little to do with the incidence of experimental carcinoma, except that a certain number of animals, nursed by mothers susceptible to mammary cancer, died of mammary cancer before the age at which cervical cancer appears.

The high incidence of cervical cancer in these experimental groups emphasizes that estrogen is a very important factor, not merely an incidental one, in cervical carcinogenesis.

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Tumors Produced by Estradiol Benzoate in the Guinea Pig

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Nelson (4) injected 32 guinea pigs with various estrogens for periods ranging from 2 to 10 months, and in 6 of these animals succeeded in producing in the cornua or fundus of the uterus one or more growths which appeared to be definitely fibromyomatous. Lipschütz (2) and collaborators, in an extended series of experiments, produced similar tumors not only on the uterus but on many of the abdominal organs, including the stomach, spleen, and omentum. He noted that ovariectomized guinea pigs almost invariably developed growths after the administration twice weekly for 2 to 3 months of 80 mgm. of estradiol benzoate. The tumors that resulted were not observed to undergo malignant change.

The present experiments were designed to determine whether the uterus played a part in the production of tumors of other abdominal viscera.

Method

The experiments were divided into two groups. In the first, both ovaries and the uterus, including the cervix, were removed in each of 9 female guinea pigs. After at least 3 weeks, when the animals were entirely recovered, a 50 mgm. pellet of estradiol benzoate was inserted just beneath the skin of the right flank. This was allowed to remain in place until the termination of the experiment, a period varying from 105 to 133 days.

A second group of 3 male guinea pigs received estradiol benzoate pellets in the same manner, without preliminary operative procedure. Two pellets weighed 25 mgm., the third 50 mgm. One of the animals with a 25 mgm. pellet was allowed to live 90 days, the other two were kept for 148 days and then killed.

Results

Each animal of the first group had well over a hundred small, firm, white, translucent nodules of varying size; the majority were sessile, a few pedunculated (Table I). Each nodule was roughly spherical and measured from less than 0.5 mm. to 2 cm. in diameter. The tumors were scattered irregularly over the peritoneal surface (Fig. 1). The sites of predilection seemed to be the surface of the spleen, stomach, and adjacent diaphragm, the terminal ileum, and omentum. Careful examination revealed no similar changes either on the other serous surfaces, including the pleura and pericardium, or in the parenchymatous tissues.

Table I: Bilaterally Oophorectomized, Hysterectomized Guinea Pigs Receiving Pellet of Estradiol Benzoate Subcutaneously

Guinea pig	Estradiol benzoate mgm.	Period in days	Site of tumor						
			Vagina	Stomach	Spleen	Diaphragm	Intestine	Parietal peritoneum	Mesentery
5	50	105	+	—	+	—	+	—	—
7	50	105	.5 cm.	+	+	+	+	—	—
8	50	126	1.5 cm.	+	+	+	+	+	+
9	50	126	—	+	+	+	+	+	+
10	50	123	—	+	+	+	+	+	—
12	50	127	—	+	+	—	+	—	—
15	50	105	.75 cm.	+	+	+	+	+	—
16	50	133	—	+	+	+	+	—	+
17	50	133	2 cm.	+	+	+	+	—	+

+ = presence of tumor; — = no tumor present.

The vaginal cuff in four instances was the site of a very much larger nodule than was found elsewhere. These were directly associated with the suture used in closing the cuff. On section, three of them were found to have small central abscess pockets which drained into the vagina.

The pellets, found to be almost their original size,

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were encapsulated by a thin layer of connective tissue. Beyond this no gross sign of local reaction was seen. The musculature beneath the pellets showed no reaction, and the underlying peritoneum showed no greater tendency toward tumor formation.

FIG. 1.—Tumors on the serosal surface of the stomach. Mag. × 2.5.

the only indication of change in the underlying tissues. Some tumors were typical fibromyomas, the fibrocytes and muscle cells differentiating clearly with Masson's stain. Muscle fibrils could also be demonstrated with phosphotungstic acid hematoxylin. The great majority of small nodules, however, presented a somewhat different picture. There were no elements within them which stained specifically for muscle. The nuclei of the cells were narrow and ribbon-like, and the abundant intercellular tissue stained green with Masson's stain, indicating its collagenous nature. These would have to be classified as fibromas.

Many of these nodules had a third element within them which was somewhat confusing. This consisted of large cells with vesicular nuclei; and with cytoplasm which was colored more or less pink with Masson's stain. Some of these cells were occasionally caught in division. These cells occurred in clusters in some areas, while in others they appeared to line ducts and crevices within the tumor. When the ducts were traced to the surface it could be seen that these cells covered the tumor. They were often arranged several layers deep, and at times were markedly hypertrophied. Projecting from the surface as columnar-like epithelium, their free ends were distended to give the whole a tear-drop appearance (Fig. 2). This was particularly marked at the edge of the tumor where the cells were not spread out and a fairly steep gradation to the usual flat serosal cell type

TABLE II: MALE GUINEA PIGS RECEIVING ESTRADIOL PELLET SUBCUTANEOUSLY

Guinea pig	Estradiol benzoate mgm.	Period in days	Site of tumor						
			Testes	Stomach	Spleen	Diaphragm	Intestine	Parietal peritoneum	Mesentery
20	25	148	—	—	—	—	—	+	—
23	25	90	—	—	—	—	—	—	—
29	50	148	+	+	+	+	+	+	+

+ = presence of tumor; − = no tumor present.

Among the second group, (Table II), nothing remarkable was found in one guinea pig exposed to a 25 mgm. pellet for 90 days. The second animal, 148 days after the pellet had been implanted, showed only a few small nodules on the lateral abdominal wall. However, the third, which had harbored a 50 mgm. pellet for 148 days, had several hundred small nodules entirely similar to those of the first group. Here, in addition to the usual sites, many small nodules were present on the serosal surface of the testes.

It should be emphasized that similar nodules have not been found in any of the many guinea pigs autopsied during the course of various experiments in the laboratory.

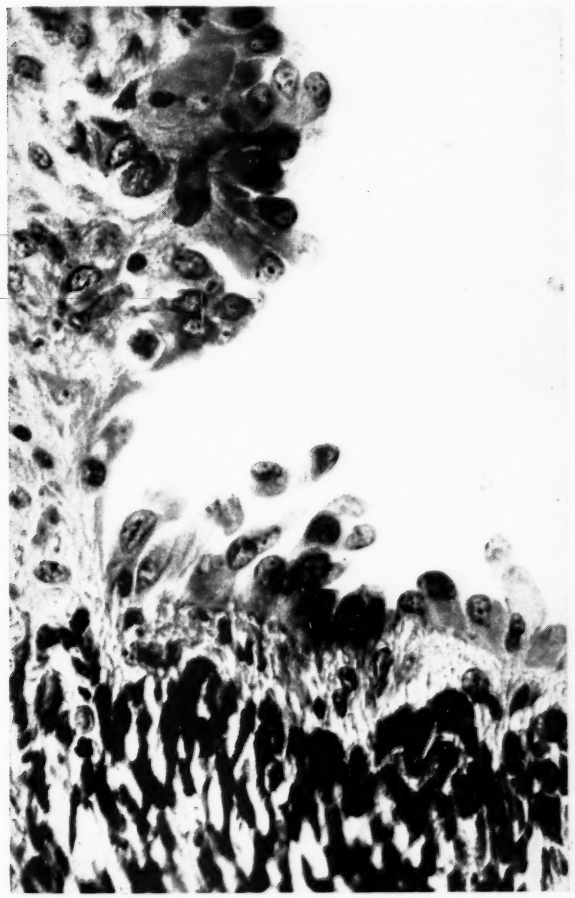
Histologically, all of the tumors appeared to originate from the serosal or subserosal layer of the peritoneum. They projected into the cavity, atrophy being occurred in a very short distance from the tumor. The relation of these cells to the serosal cell has not been established, but it is clear that they continued into the above-described crevices, and appeared as acinar-like masses within the connective tissue stroma of the nodules.[1] Both in the crevices and in the acinar-like arrangement, these cells at times formed several layers, and again showed evidence of growth on the surface (Fig. 3). Efforts to demonstrate muscle fibrils within them met with failure.

Around the central portion of three growths on the vaginal cuff there was a fibrous wall infiltrated by a small number of polymorphonuclear leucocytes.

[1] Since this work was completed, Lipschütz and Vargas (3) have noted an intimate relation between the cells of the serosa and certain cells of the tumor. This is in agreement with the present findings.

This was surrounded by a much larger mass of quite young granulation tissue which closely resembled the majority of the tumors in structure.

In sections, the positions of the pellets of estradiol benzoate were indicated by a nuclear free matrix, from which the estrogen had been dissolved by the dehydrating agents. This was surrounded by a thin layer of connective tissue. Within this tissue were many vacuolated giant cells, particularly near the



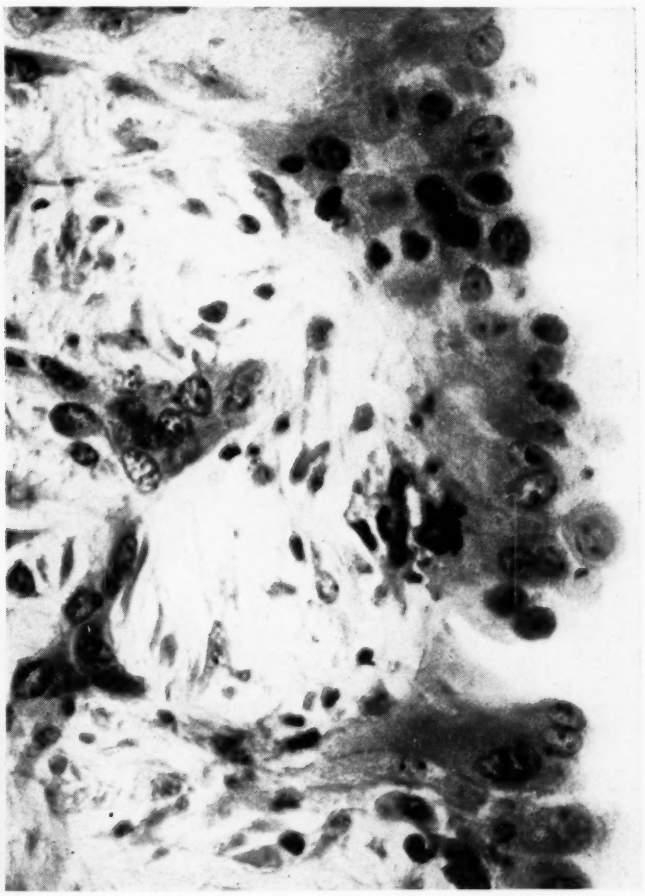


FIG. 2 (Left).—Showing the marked hypertrophy and hyperplasia of the serosal covering cells of a tumor. Mag. × 150.
FIG. 3 (Right).—Duct-like extensions or inclusions composed of hyperplastic serosal cells. Mag. × 300.

periphery, while strands of capillaries and granulation tissue ramified further into the matrix.

In all except one female, the vaginal mucosa showed the large foamy cells typical of estrogenic stimulation. In this animal no pellet could be found at autopsy. It was therefore assumed that the pellet had sloughed out. Small nodules remained on the peritoneum, however, which had been produced while the pellet was in place.

DISCUSSION

The high incidence of these tumors, 100 per cent in the first group of 9 guinea pigs, together with their multiplicity, sites of origin, and the constancy of involvement of these sites, is significant and substantiates the previous work of Lipschütz.

The absence of uterine tissue seemed to have exerted no effect on the production, frequency, or distribution of tumors resulting from estradiol benzoate.

Why these tumors should have such a widespread distribution throughout the abdominal cavity, but be absent from all other parts of the body, is not clear. The tumors consisted of proliferating fibrous tissue and occasionally muscle tissue. Structures interpreted as the participation of serosal cells in the tumor are a prominent and highly peculiar feature. The relation of this hyperplastic cell to the formation of the tumor requires further investigation.

Three of the four large tumors found on the cuff of the vagina appeared to have been formed about an abscess pocket. Since these three were composed particularly of granulation tissue, the question is raised whether they may represent estrogenic stimulation of granulation tissue. The abdominal scars showed no tendency toward keloid formation, but this is hardly

to be expected since the wounds were well healed before the pellet was introduced.

Summary and Conclusions

1. Ovariectomized and hysterectomized female guinea pigs and a male guinea pig which received a 50 mgm. pellet of estradiol benzoate subcutaneously developed tumors in the peritoneal cavity in 100 per cent of the cases.

2. Muscle fibers were found in only a small number of these tumors; the majority of tumors appeared to be fibromas.

3. The absence of uterine tissue had no effect either on the frequency or distribution of these tumors.

4. The serosal cells seemed to participate in the formation of the tumor. They occurred as small aggregations of actively growing cells where the tumor and serosa joined, and also lined crevices and extended into the tumor as pseudo-acinar structures.

5. An extensive overgrowth of granulation tissue, histologically similar in many respects to the tumors, occurred in relation to three abscess pockets.

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The Relation of Diet to Benign Neoplasia (Ulcero-Papillomas) of the Rat's Stomach*†

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The experiments reported in this paper were designed to investigate the relation of dietary factors to carcinogenesis in the stomach of the rat.

In 1913 Singer (17) called attention to ulcero-papillomatous lesions in the forestomach of rats due, he felt, to infection by *Spiroptera* because of Fibiger's (7) similar observations made at the same time but regarded by the latter as carcinomas resulting from infection by these parasites. Later Pappenheimer and Larimore (13) observed the lesions in rats fed a rickets-producing diet, and held that they were the result of some dietary deficiency, but not Vitamin A. They felt that some mechanical traumatic factor might contribute to the development of these lesions since wood shavings and hair were often found in the affected stomachs. Hoelzel and Da Costa (10) produced the lesions in both the forestomach and glandular portion by partial starvation and by feeding a diet low in protein. These authors do not appear to have differentiated the ulcero-papillomatous lesions from acute or more chronic flat erosions or ulcerations of the mucosa with little or no marginal proliferation. Sharpless (16) likewise observed ulcero-papillomas in the rumen in rats fed a diet containing only 4 per cent casein, and reported their absence with a diet containing 25 per cent casein or 4 per cent casein to which cysteine and methionine were added. One group of investigators attributed the lesions to vitamin deficiency. According to Fujimaki *et al.* (9), Santi (15), Bisceglie (1), Fredericia (8), and Cramer (4) it is a question of lack of Vitamin A, although Wolbach and Howe (18) did not observe them in their experiments on Vitamin A deficiency. Dalldorf and Kellogg (5) observed shallow mucosal erosions and apparently ulcero-papillomas in both portions of the stomach in rats fed Vitamin B_1 deficient diets. Howes and Vivier (11), who have published an excellent review and discussion of the whole subject, confirmed the observations of Pappenheimer and Larimore and further noted that whole yeast afforded relative protection. They made the important observation that the lesions were more easily developed in young than in adult rats. Roffo (14) in a great number of experiments observed ulcero-papillomas in the stomachs of rats fed heated animal fats in addition to the stock laboratory diet of milk, bread, and greens. He attributed the proliferative lesions to carcinogenic potencies of the heated fats. Domagk (6) reported the development of an "adenocarcinoma" in the stomach of one mouse and papillomas in others, fed rice and olive oil for weekly periods alternating with weekly periods of regular diet for a year. Büchner, Siebert, and Molloy (2) and Bürkle-de-la-Camp (3) produced the lesions in rats by means of partial starvation and subcutaneous injections of histamine. These authors are of the opinion that the lesions were due to the corrosive effects of the gastric juice which could be augmented by starvation and histamine injection. Matzner, Windwer, and Sobel (12) ascribed the development of the lesions in rats fed low protein diets to the lack of protection from the proteolytic action of gastric juice. It was claimed that high protein diets containing casein, gelatin, and elastin afford this protection.

From the above incomplete, but representative review, it appears that the lesions in question have been observed under a variety of conditions. This suggested a further investigation from the standpoint of a possible nonspecific etiology; *i.e.*, a lesion that represents in the rat a reaction resulting from a diet inadequate in one or more components, or groups of dietary factors.

Experimental

The animals employed were young white rats of both sexes, weighing 60 to 130 gm., from the colony inbred in this laboratory for the past 8 years.

In some instances groups of 5 or more animals were put together in large cages. Where it was anticipated that the animals would not eat much of the diet and therefore would be likely to kill and devour each other they were put in individual cages. Water was available in unrestricted quantities in all instances.

Controls.—In order to control the adequacy of the laboratory stock diet of white bread, lung stew, lettuce, and carrot greens, insofar as affording animals with normal stomachs is concerned, 16 rats over 2 years of age, reared in the colony and kept in various cages in the same room, were killed and the viscera examined. The stomach and other viscera were found to be normal.

Low protein and high fat and carbohydrate diets.—The results are summarized in Table I. In four groups the animals were weighed at the beginning of the experiment and at death. The loss of weight was most marked in those fed lard only. This group also had diarrhea. Partial starvation was also a factor in the

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‡ Research Assistant, The International Cancer Research Foundation Grant.

lard group since they ate very limited quantities. Boiled rice was eaten more readily and there was no diarrhea. The animals died spontaneously or were killed by a blow on the head. Those dying earlier than the minimum period indicated are not considered. In all the following tables animals are considered "positive" in which one or more typical ulceropapillomas or papillomas were present in the rumen or glandular portion of the stomach. The lesions, if present, were usually multiple.

In group D (Table I), the vitamins were administered by placing one drop of Vipenta Oil-Roche in each animal's mouth three times a week. This was readily swallowed. According to the manufacturer one drop contains approximately 1,000 U.S.P. units of Vitamin A, 20 international units of Vitamin B_1, 10 γ of riboflavin, 45 international units of Vitamin C, and 100 U.S.P. units of Vitamin D. The gain in weight registered in this group might well be the result of appetite stimulated by the vitamin intake (B_1). In spite of the gain in weight however proliferative gastric lesions developed.

Weights are not recorded for groups E and F (Table I) as these were adult animals having attained full development, whereas the rats employed in the other group of experiments were immature. These rats, however, obviously exhibited a moderate weight loss.

High protein diet.—Since importance has been attached to the protein content of the diet as regards the etiology of the lesions (10, 16), another series of rats was fed high protein diets. The results are summarized in Table II. Where casein alone was fed to 18 rats, 9 of the 10 surviving 3 or more weeks exhibited no lesions and in the one "positive" stomach the lesions were quite small. The remaining 8 did not survive more than 5 to 8 days (1 for 14 days), exhibiting loss in weight up to 40 per cent. At necropsy these animals showed multiple pin-point and streaked hemorrhagic erosions of the gastric mucosa often with hemorrhagic fluid in the stomach and bowels. These rats, which had been kept in separate cages, were observed to have partaken of only very small quantities or practically none of the casein diet and thus in reality died of starvation. In group B (Table II) where the diet consisted of equal parts of casein and lard (heated to 220°C.), the incidence of the lesions was over 50 per cent with considerable loss in weight. The loss of weight, however, was not greater than in group A (Table II) (casein only) where the incidence of lesions was much lower. In groups C and D (Table II), the weight remained stationary in group C

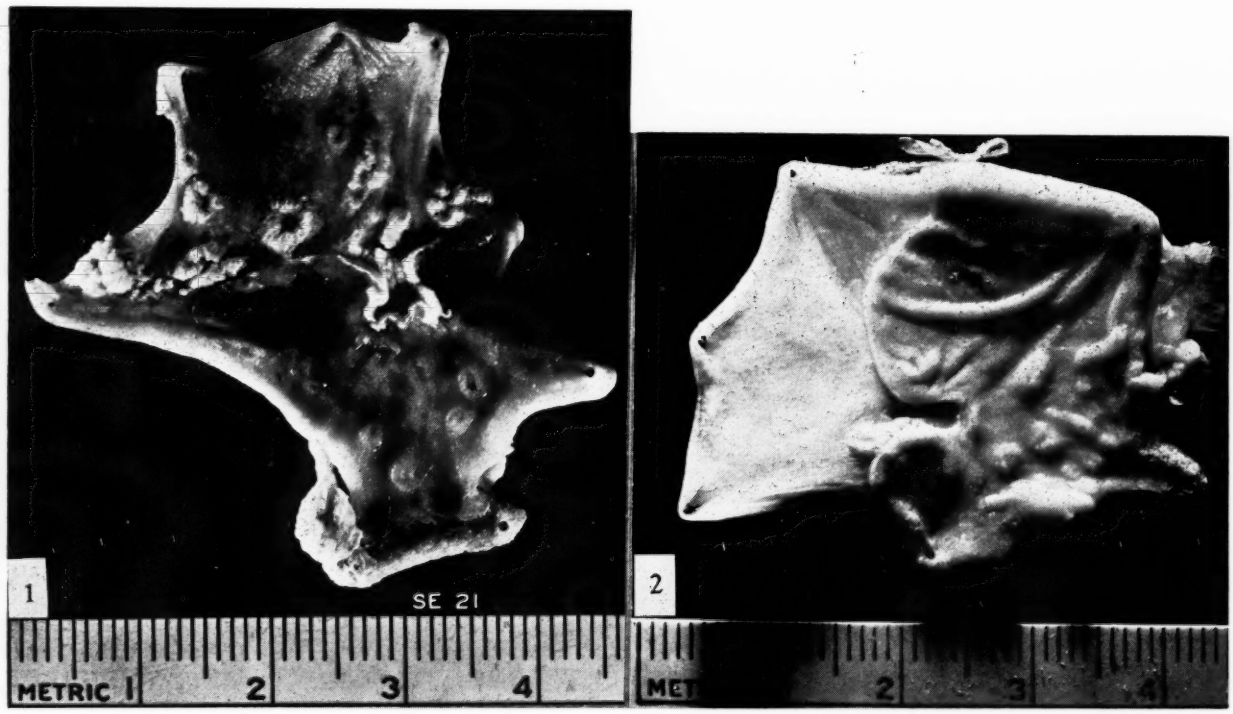


FIG. 1.—Stomach of rat fed boiled polished rice and lard (equal parts) for 175 days. Sessile papillomas and ulcero-papillomas in both rumen and glandular regions of stomach.

FIG. 2.—Stomach of rat fed equal parts of boiled rice and lard previously heated to 220° C. for 30 minutes, for 141 days. One ulcero-papilloma low in rumen and multiple proliferative lesions in glandular portion, lesser curvature.

but there was an appreciable loss in group D, yet a low incidence of lesions was observed in each group. This series of experiments would indicate that weight loss *per se* is not the sole or even principal etiologic factor in the formation of the proliferative lesions.

Brewer's yeast diets.—A series of experiments was conducted in which brewer's yeast, moistened with water to make a paste, was the only food offered. As with other unbalanced diets, some of the animals ate sparingly if at all, whereas others ate sufficient quantities to maintain or gain slightly in weight. It was noted than when animals were placed in small individual cages, they usually ate little and died in a few days; whereas in larger cages with other animals the individuals seemed to eat more and fare better. The results are summarized in Table III. The 21 rats fed yeast only are considered in two groups: one in which the animals died within 7 days, and the other in which the animals survived 11 to 38 days. The latter appeared to have abdominal distention after a few days on the pure yeast diet and at necropsy this was found to be due to large amounts of soft fecal material in the colon and appendix. It is of interest to note that with yeast alone or combined with casein no proliferative lesions developed, the animals maintaining or gaining slightly in weight, whereas, on diets of lard (85 per cent) and yeast (15 per cent), the amounts ingested were so limited as to lead to marked loss of weight accompanied by the development of proliferative lesions. On the other hand, yeast alone if eaten in sufficient quantities to afford a stationary or slight gain or loss in weight seems to protect against the lesions; casein alone also affords a high degree of protection since, as shown previously, the incidence of the lesions is very low even though there is marked weight loss. It is thus again suggested that nitrogenous foods (proteins) afford relatively greater protection against the lesions, even though emaciation develops, than non-nitrogenous foods such as fats and carbohydrates. In group F (Table III), 13 animals were fed boiled rice and yeast (15 per cent of total volume) with maintenance of weight for 52 days. The development of proliferative lesions in 6 instances again illustrates that emaciation *per se* is not an important etiologic factor.

TABLE I: SUMMARY OF INCIDENCE OF ULCERO-PAPILLOMAS AND PAPILLOMAS OBSERVED IN RUMEN AND GLANDULAR PORTION OF STOMACH IN RATS FED HIGH CARBOHYDRATE AND HIGH FAT DIETS

Diet	No. of rats	Duration of feeding, in days	No. of animals with lesions	No. of animals without lesions	Average weight, onset of experiment (grams)	Average weight, end of experiment (grams)
A. Lard only, previously heated to 220° C.	6	18-29	4	2	97	44
B. Unheated lard	11	18-30	4	7	Animals lost weight	
C. Boiled polished rice	8	30-43	6	2	130	85
D. Boiled rice and vitamins A, B, B_2, C, D	8	35-43	4	4	80	101
E. Boiled rice and unheated lard—equal parts	9	90-182	6	3	..	..
F. Boiled rice and heated lard—equal parts	10	122-160	8	2	..	..

TABLE II: SUMMARY OF INCIDENCE OF ULCERO-PAPILLOMAS AND PAPILLOMAS OBSERVED IN RUMEN AND GLANDULAR PORTION OF STOMACH IN RATS FED A HIGH PROTEIN DIET

Diet	No. of rats	Duration, in days	No. of animals with lesions	No. of animals without lesions	Average weight, onset of experiment (grams)	Average weight, end of experiment (grams)
A. Casein only	10	20-53	1	9	130	75
B. Casein and heated lard—equal parts	17	32-35	10	7	104	77
C. Casein and heated lard and Vitamins A, B, B_2, C, D	9	43-57	2	7	85	107
D. Casein and unheated lard—equal parts	6	42	1	5	94	77
E. Casein and boiled olive oil (30%)	12	32-40	4	8	90	66

Deprivation of food.—The effects on the stomach of complete deprivation of food, except for water, were observed in a series of animals. Rats of the size employed apparently could not long survive complete starvation. They died in 2 to 6 days after rapid and marked loss in weight. Subcutaneous injections of 0.5 gm. of amino acids in water derived from complete enzyme hydrolysis of casein were made daily in another series of 5 rats, which did not survive for a longer period. A third series was given the stock laboratory diet of bread, lung stew, and lettuce in markedly reduced quantities but survival was not prolonged. At necropsy all animals exhibited the same findings in the stomach. There was a varying amount of dark coffee ground hemorrhagic exudate in the lumen and the glandular mucosa presented multiple pin-point or elongated streaked hemorrhagic erosions in the glandular portion and less frequently in the rumen. The erosions were quite superficial and ap-

peared macroscopically to be confined to the mucosa. Histologic study confirmed this; the mucosa seemed to have become necrotic with interstitial hemorrhage at the involved points and there was little underlying leukocytic infiltration. The changes were not postmortem since most of the animals were autopsied immediately or a short time after death. The protocols

velop in animals which have access to unlimited quantities of the laboratory stock diet but which, because of pronounced emaciation, did not obviously partake of sufficient quantities for normal maintenance, a group of 13 rats with varying degrees of emaciation and bearing large methylcholanthrene sarcomas, some ulcerating through the skin, were killed and the

TABLE III: SUMMARY OF EXPERIMENTS ON BREWER'S YEAST DIETS

Diet	No. of rats	Duration of experiment, in days	No. of rats with gastric lesions	No. rats negative	Average weight, at onset (grams)	Average weight, at death (grams)
A. Brewer's yeast only	7	1 to 7	6—acute hemorrhagic erosions	1	79	56
B. Brewer's yeast only	14	18 to 38	1—acute hemorrhagic erosions	13	67	61
C. Casein and yeast (15%)						
a′	6	40 to 43	0	6	97	91
b′	5	42	0	5	87	105
c′	2	52	0	2	70	95
D. Heated lard and yeast (15%)	6	19 to 30	4—ulcero-papillomas and papillomas	2	98	50
E. Unheated lard and yeast (15%)	8	17 to 35	4—ulcero-papillomas and papillomas	4	103	47
F. Boiled rice and yeast (15%)	13	52	6—ulcero-papillomas and papillomas	7	75	76

TABLE IV: SUMMARY OF EXPERIMENTS ON COMPLETE AND PARTIAL STARVATION

Regime	No. of rats	Survival in days	Average weight, onset of experiment (grams)	Average weight, at death (grams)	Necropsy
A. No food	3	4	82	54	Acute hemorrhagic erosions in gastric mucosa
B. No food—daily injection of 0.5 gm. amino acids	5	3 to 6	147	97	Acute hemorrhagic erosions in gastric mucosa
C. 1 gm. of stock diet a day	3	2 to 4	65	45	Acute hemorrhagic erosions in gastric mucosa
D. 3 gm. of stock diet a day	5	2 to 4	73	56	Acute hemorrhagic erosions in gastric mucosa
E. 4 gm. of stock diet a day	4	7	133	85	Acute hemorrhagic erosions in gastric mucosa
F. 5 gm. of stock diet a day	6	3 to 4	82	52	Acute hemorrhagic erosions in gastric mucosa
G. 6 gm. of stock diet a day	7	3 to 6	74	47	Acute hemorrhagic erosions in gastric mucosa
H. 2 gm. of stock diet a day + subcutaneous injection of Vitamin K, .8 mgm. a day	4	1 to 3	90	59	Acute hemorrhagic erosions in gastric mucosa

are summarized in Table IV. In no instance were there ulcero-papillomas or papillomas of the type referred to in the preceding sections. To rule out the possibility of such lesions being the result of Vitamin K deficiency, 4 rats were given 2 gm. of stock diet a day and received daily subcutaneous injections of 0.8 mgm. of Vitamin K. They died in 1 to 3 days with typical hemorrhagic lesions.

In order to observe whether or not lesions may de-

stomachs examined. In 4 there were typical ulcero-papillomas in the rumen and glandular portion; in 1 there was an oval chronic ulceration 2 x 1 mm. without raised edges; in the remaining 8 there were no gastric lesions. The stomachs exhibiting lesions were from animals which exhibited the most marked degree of emaciation. These animals had all been kept in cages with nontumor-bearing animals and had access, as stated above, to unlimited amounts of the stock diet.

Pathology of the Lesions

There appears to be some confusion in the literature regarding the pathologic terms employed in describing the lesions in question. The problem has been further complicated by the fact that the proliferative type of lesion has been observed together with flat shallow ulcerations with only slightly or no elevated borders. In addition, acute pin-point and streaked mucosal hemorrhagic erosions have also been observed. The latter lesions are essentially acute ulcerations and do not represent the same type of tissue reaction that is exemplified in the ulcero-papillary and papillary lesions.

The ulcero-papillomas in the rumen consist of well circumscribed sessile lesions, rounded or oval in shape, with long axes in any direction and situated more frequently near the border between rumen and glandular portion than high in the fundus. In the series observed by the authors they were also most frequently observed in the rumen opposing the esophageal orifice. The borders are raised one or more millimeters above the mucosal surface. In the central portion surrounded by the borders is an ulcer crater of varying depth. This crater usually does not exceed a third of the diameter of the whole lesion and is often less than this. Histologically the elevated borders are seen to consist of a papillary proliferation of the stratified squamous epithelium, often with considerable hyperkeratosis. While there is also a "rete-peg"-like downgrowth of the epithelium comprising the lesions, there was no

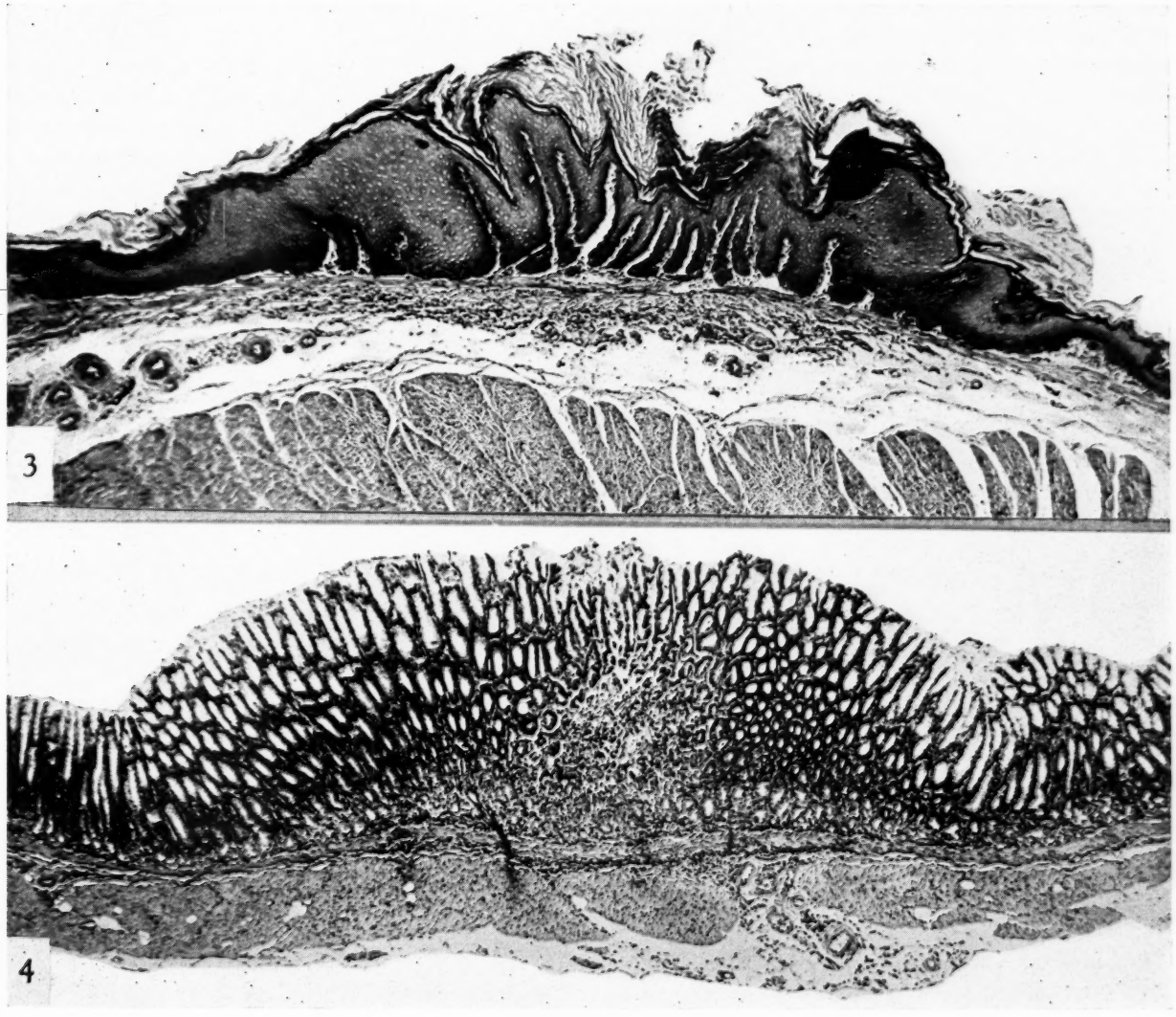


Fig. 3.—Photomicrograph of section (× 55) through sessile squamous cell papilloma in rat's forestomach produced by inadequate diet (see text).

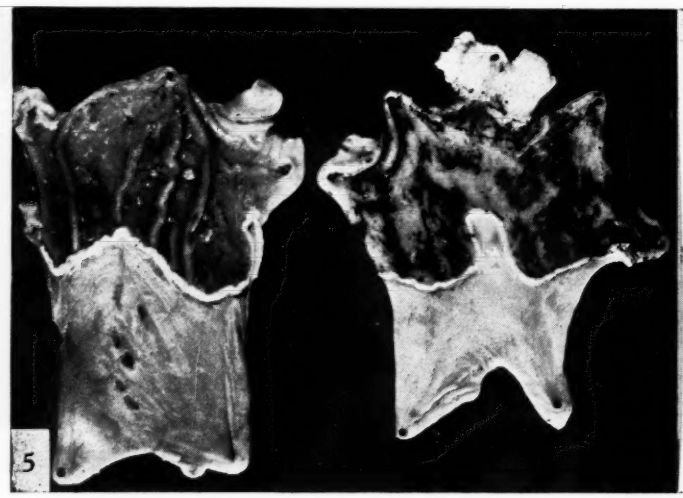
Fig. 4.—Photomicrograph of section (× 55) through sessile polypous lesion in glandular portion of rat stomach produced as above. Beginning necrosis in central portion to produce umbilicated ulcer. The process does not begin on the surface and extend downward, but arises within the lesion itself.

suggestion in any instance of unrestrained proliferation of cell masses into the submucosa or muscularis which would exist if the lesions were malignant. The individual epithelial cells appear larger than normal, the nuclei more vesicular, and they exhibit frequent mitotic figures. There is round cell and polynuclear infiltration in the underlying submucosa and muscularis where there is also edema and an increase in blood capillaries which are for the most part dilated with blood. The ulcer crater varies in depth and may extend only partially through the thickened stratified squamous epithelium in which case cells of the latter form its floor; or it may extend to the submucosa where infiltrated connective tissue of the latter forms the base. The crater is filled with leukocytes, debris, and desquamated keratin fragments.

In addition to the ulcero-papillomas, sessile papillary lesions without ulceration are observed. These have the typical gross and microscopic appearance of squamous cell papillomas. In some instances they appear to be confluent, in which case rather extensive segments of the rumen exhibit a roughened, raised, shaggy appearance. Confluence of papillary and ulcero-papillary lesions are also observed.

In the glandular portions of the stomach the proliferative lesions which were usually situated along the lesser curvature appear as rounded or oval disc-like elevations of the mucosa 1 to 2 mm. in diameter with, in some instances, apparent intact smooth superficial surfaces but more often with a minute ulceration umbilicating the central portion of the lesion. On histologic study these lesions are seen to be due to an elongation of the glands of the gastric mucosa. The individual cells appeared somewhat larger than normal in the basal portions and there are rather frequent mitotic figures. Here again there was no instance of unrestrained proliferation of cell masses into the submucosa and muscularis. In the latter regions the inflammatory reactions were essentially as described above. The small ulcer craters also varied in depth and were filled by cell detritus. No instance of metaplasia into squamous epithelium was encountered. Confluence of separate lesions in this region was not observed.

In the opinion of the writers the proliferative lesions just described are not different stages of the same process of which the flat ulcerations and erosions might be regarded as another stage. There was no evidence upon examination of stomachs after varying periods on different diets and the observations of lesions of different sizes that one type developed into the other. On the other hand, it is of course possible that the simple ulcers and the proliferative lesions may have some etiologic factors in common.



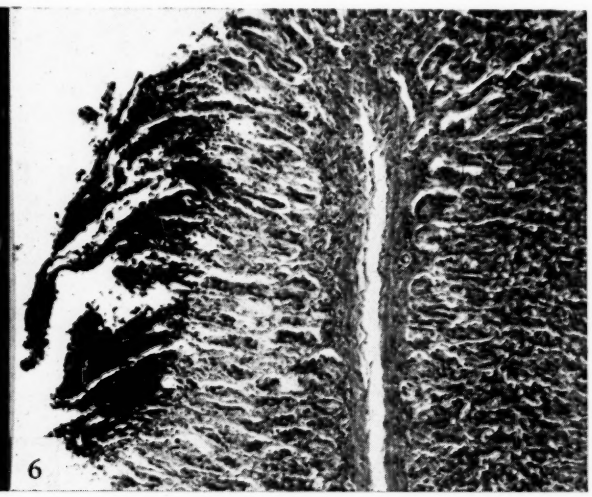


FIG. 5.—Acute mucosal hemorrhagic lesions in glandular portion of stomachs and oval shallow ulcers in rumen of one, observed in rats deprived of all food except water and dying on third day of starvation.

FIG. 6.—Photomicrograph (× 125) of a lesion shown in Fig. 3. Note superficial hemorrhage into mucosa and absence of leukocytic infiltration.

DISCUSSION

In the experiments reported above the proliferative type of gastric lesion was observed in rats fed a variety of unbalanced diets such as fat exclusively, high carbohydrate as exemplified by rice (8 per cent protein, 79 per cent carbohydrate), and high protein diets such as mixtures of equal parts of casein and fat. It would thus appear that there is no specific type of inadequate diet which constitutes the principal etiologic factor. As shown in the tables, where the incidence of the proliferative lesions was appreciable there was loss in weight. There are exceptions, however, since in the

group fed casein only the loss of weight was up to 40 per cent, yet only 1 of 10 animals developed lesions. In groups C and D (Table II), the incidence of lesions was the same for each group, although in the former, which received casein and heated lard and vitamins A, B_1, B_2, C, and D, the weight remained stationary while in the latter group receiving the same diet without vitamins the loss of weight was about 20 per cent. Furthermore in group D (Table I), where boiled rice with vitamins constituted the diet, there was a 20 per cent gain in weight but 4 of 8 rats developed the proliferative lesions.

It would also appear that a low protein diet *per se* could not account for the lesions. In group B (Table II), the diet contained 50 per cent protein yet the incidence of the lesions was high. The weight loss, however, would indicate that the food was not ingested in large amounts. Thus the factor of quantity of food eaten also appears to be of etiologic importance. The significantly low incidence of lesions in rats fed casein only might well be due in part to the presence in the stomach of a pasty bolus (usually found in these animals) with acid neutralizing properties as emphasized by others (2, 3, 12).

The lesions do not appear to be the direct result of deprivation of vitamins A, B_1, B_2, C, or D, nor do they seem to be due to a carcinogenic-like action of heated fats (as inferred by Roffo). Where radical restriction of food was obtained, the animals of ages employed in these experiments died in a few days with hemorrhagic mucosal ulcers and erosions, a type of lesion apart from the proliferative type as discussed above and also not the result of Vitamin K deficiency. Such hemorrhagic lesions were also characteristic of stomachs of rats deprived entirely of food, but allowed water.

In the groups fed diets containing yeast, proliferative and hemorrhagic lesions were observed. The fact that one group received brewer's yeast alone for 3 weeks or longer (one dying in 11 days with hemorrhagic erosions of the stomach) and exhibited slight gain in weight and no proliferative lesions would indicate that yeast afforded a certain degree of adequate nourishment. All animals receiving casein (85 per cent) and yeast (15 per cent) fared rather well and exhibited no lesions, the majority of the rats gaining some weight. On the lard (85 per cent) and yeast diets (15 per cent) weight loss was marked indicating limited ingestion of food. In these groups the lesions that developed were ulcero-papillomas and papillomas, not of the hemorrhagic erosion type, indicating that when combined with lard and ingested in small quantities yeast could not afford protection against these lesions.

In the opinion of the writers, the data presented in this series of experiments suggest that when a certain degree of malnutrition is developed in the rat, such malnutrition not necessarily being synonymous with absolute loss of weight nor with the lack of any specific type of food or vitamin, the mucosa of both the rumen and glandular portions of the stomach react by localized foci of cellular proliferation. These lesions exhibit the characteristics of benign neoplasia. There is, of course, the possibility of a lack of some as yet unidentified vitamin.

"Cure" of the proliferative lesions.—To obtain further evidence in support of the last statement, 16 rats were divided into two groups of 8 each. One was fed casein and boiled olive oil (15 per cent by weight) for 40 days; 4 were then killed and all showed proliferative gastric lesions. The remaining 4 were fed the regular stock diet for 16 days and when killed were found to have normal stomachs. The second group received boiled rice only for 67 days; 4 were then killed and each showed proliferative gastric lesions. The remaining 4 were then fed the stock diet for 13 days and when killed had normal stomachs.

Summary and Conclusions

Gastric lesions develop in rats as a result of a certain degree of malnutrition or possibly as the result of a deficiency of some as yet unidentified vitamin.

1. Deprivation of food or marked curtailment in quantity of an adequate stock diet results in death in a few days with multiple pin-point and acute streaked hemorrhagic erosions and ulcerations in the glandular portions of the mucosa and, to a lesser extent, in the rumen of the stomach. Such lesions might be regarded as a characteristic "starvation reaction" in the young rat's stomach. They are not due to inadequate quantities of Vitamin K.

2. When any one of a variety of unbalanced diets is given which permits survival for three or more weeks, usually, but not always with marked loss in weight, proliferative lesions develop in both regions of the stomach which have been designated as ulcero-papillomas and papillomas and which have the histologic characteristics of benign neoplasias. Such lesions might be regarded as a type of "chronic malnutrition" reaction in the rat's stomach representing a certain degree of inadequate nourishment.

Pure protein (casein) diet appeared to avoid the development of the lesions. Pure brewer's yeast diets when eaten in sufficient quantities to permit maintenance or result in slight loss in weight also appeared to protect against the formation of the gastric lesions. Diets high in protein or with 15 per cent of yeast, especially if eaten sparingly, do not necessarily protect against the lesions.

3. No evidence was obtained to indicate that a lack

of either vitamins A, B_1, B_2, C, or D was an important direct etiologic factor in development of the proliferative lesions.

4. No evidence was obtained to indicate that the proliferative lesions were induced by a carcinogen-like action of a high cholesterol (lard) component in a diet whether or not the fat was heated to 220° C.

5. Evidence was obtained to indicate that when the proliferative lesions are developed as a result of unbalanced diet, a resumption of full adequate diet leads to a disappearance of the lesions in about 2 weeks.

6. In experiments designed to investigate carcinogenesis in the stomach in rats, it is obvious that the dietary factor (nutritional state) must be fully controlled.

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The Transmission of an Induced Lymphatic Leukemia and Lymphosarcoma in the Rat*

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Evidence accumulated during recent years has made it progressively more evident that the essential factors in neoplasia and leukemia vary only in the type of cell involved in the malignant process. The attempts to produce leukemia experimentally by chemical carcinogenic agents have succeeded with some frequency in mice (3, 4, 10). As the spontaneous disease is not uncommon in these animals, Richter and MacDowell (13) have cautioned against overlooking the influence of genetic variables in the claims for the successful inductions. Certainly in some of the strains used for these studies, leukemia is known to occur spontaneously, as pointed out by Mider and Morton (9). However, these latter investigators leave little doubt that the carcinogenic chemicals at least bring out the disease at an earlier age and in a higher percentage of individuals than would occur spontaneously. It would thus appear that induced leukemia is probably another example of the part played by genetic constitution on the type of response which may take place to carcinogenic agents, a fact first demonstrated in this laboratory by Lynch (7, 8).

The present report has to do with the induction and transmission of leukemia in rats. This appears to be a rare condition, for among 489 tumors observed by Bullock and Curtis (2) in autopsies on over 2,400 rats, there were a number of thymus and lymph node tumors but no definite instance of leukemia. In a recent report (12) on the large Wistar Institute rat colony, only 2 out of 273 tumors which have occurred in this strain were of the lymphoid system and no cases of leukemia were mentioned. As the Wistar strain was used in the present study, the absence of leukemia and very low instance of lymphoid tumors is of particular interest. Ito (6) reports a single instance of myelogenous leukemia in the rat which he considers to have been induced by feeding amidoazo-toluol.

Method.—In the course of a study of the effect of different solvents on the carcinogenic property of 1,2,5,6-dibenzanthracene [1] 10 rats of the Wistar strain were injected in both groins with 0.5 cc. of a 3 per cent suspension of the chemical in benzol. This was followed 6 weeks later by a second injection in the same region. After another 6 weeks one rat was found to have a mass in the left groin, 1.2 × 0.7 cm. in size. A biopsy specimen showed a structure resembling a lymph node impregnated with crystals of dibenzanthracene. Two weeks later a mass was observed in the other groin. The blood count at this time showed 32,000 white cells per cu. mm., 91 per cent of which were mononuclears of the lymphoid type. A week later the white cell count had increased to 43,000 at which time the animal was killed and the blood collected from the heart to be used for an attempt at transmission.

The autopsy showed, besides the masses in the groins, marked enlargement of the lymph nodes of the axilla, neck, and peritoneum. There was no evidence of involvement of the liver, spleen, or other organs. Crystals of dibenzanthracene were found in both masses from the groins. Histologically the involved nodes were composed of closely packed lymphoid cells with disruption of the normal architecture.

Two other rats from the group developed local spindle cell sarcomas which were easily transmissible to other rats.

Transmission of leukemia.—The defibrinated blood from the original leukemia animal was injected into 7 Wistar rats of about 65 gm. in weight; 0.2 cc. in the subcutaneous tissue of the groin and 0.4 cc. intraperitoneally. The involved lymph nodes were finely minced, suspended in normal saline, and inoculated into 7 rats of the same strain. These received 0.2 cc. of the suspension subcutaneously and 0.6 cc. intraperitoneally. By the fourth week 3 of the 7 rats inoculated with blood, and 4 of the 7 injected with the cell suspension had developed typical leukemia which progressed to the death of the animals. This was characterized by a marked increase in the circulating lymphocytes and massive enlargement of the lymph nodes. The thymus in all of the affected animals was so large that it caused pressure on both the lungs and heart but, as with the original animal, there was no evidence of involvement of the other organs.

In subsequent generations both the number of takes

* This investigation was aided by a fund for leukemia studies contributed anonymously.

[1] An extensive study in progress in this laboratory has shown that dibenzanthracene in benzol gives rise to tumors in chickens in a far shorter period than when the same amount of the chemical is administered in lard or chicken fat.

and the severity of the disease has increased. In the first five transfers when young Wistar rats were used, the takes were from 60 to 80 per cent and the average length of life after inoculation was over 2 weeks, while in later generations the takes increased to between 90 to 100 per cent and the animals died in from 6 to 10 days.

Character of the disease.—The rise in circulating lymphocytes starts at about the 6th day after inoculation and progresses rapidly. A typical example of the changes in blood picture based on counts in 10 rats is shown in Fig. 1. In a number of animals surviving a little longer than the average, white counts of over

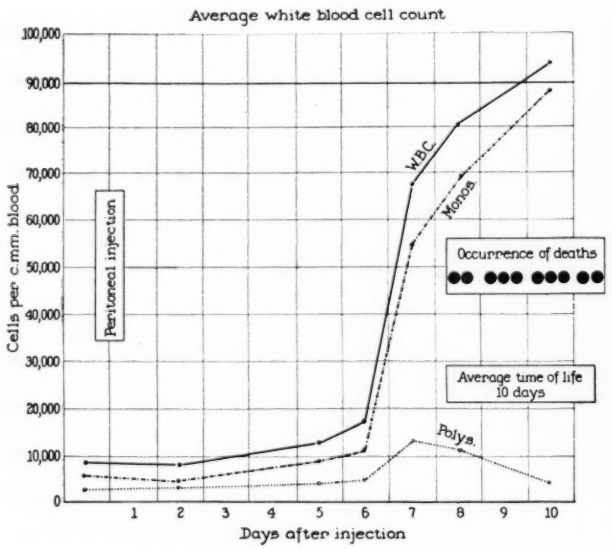

FIG. 1.—The white blood cell count following intraperitoneal inoculation of leukemia cells based on the average counts in 10 rats. The black circles indicate the time of death of the individual animals.

200,000 are not uncommon and in one instance it reached 260,000 cells per cu. mm.

Labored breathing from pressure of the thymus is noted early in the disease. There is usually a bloody discharge from the nose and about the eyes from small hemorrhages taking place in the mucosa of the nose and eyelids. At autopsy hemorrhages are noted in the involved lymph nodes, testicle, muscle, intestines, and subcutaneous tissue. All lymph nodes are greatly enlarged and the thymus is extensively involved (Fig. 2). The spleen may be slightly above the average size in some individuals but does not show a disruption of its normal structure or other evidence of involvement. The bone marrow is largely replaced by lymphocytic infiltration but other organs show no evidence of involvement.

Production of lymphosarcoma.—As noted above in the first transmissions, the rats were inoculated both subcutaneously and intraperitoneally. The resultant disease was leukemia but in a few of these animals a very thin plaque of tissue was noted at the site of the subcutaneous injection. The fate of the leukemic blood inoculated subcutaneously was next investigated.

Experiments.—Blood from a leukemic animal of the 14th generation was injected subcutaneously in 4 rats and, as controls, intraperitoneally into 4 rats. By the 14th day, 3 of the 4 in the latter group died of leukemia while none of those inoculated subcutaneously showed the disease. However, 2 of the test group developed tumors at the site of inoculation which grew to a large size, and eventually caused the death of the animals. In the course of subsequent generations some 929 rats have been inoculated sub-

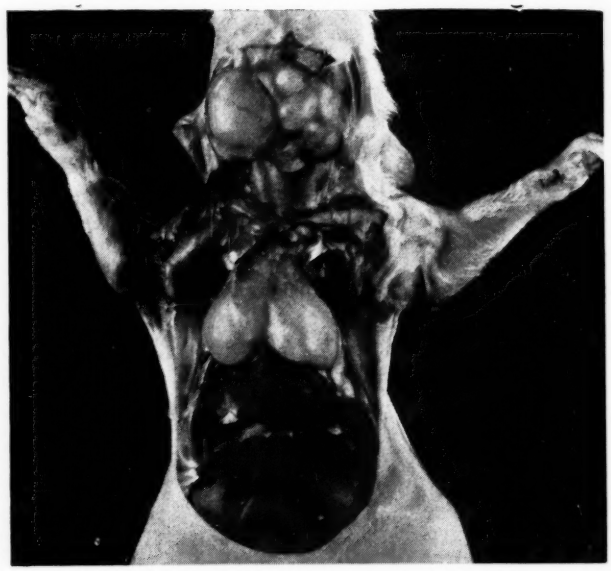

FIG. 2.—Involvement of the thymus and nodes of the neck in generalized leukemia resulting from intraperitoneal inoculations.

cutaneously with the tumor cells, leukemic blood, or involved lymph nodes. Of these, 522 have developed local tumors with no evidence of generalized disease. In later generations when there was an increase in malignancy, 7 rats inoculated subcutaneously developed local tumors and later showed leukemia. However, this continues to be of very rare occurrence. With the exception of these 7 animals the local nature of the disease produced by subcutaneous inoculation is indicated both by the fact that there is no increase in the circulating white cells of the blood and that the blood from these rats injected into normal rats fails to produce either tumors or leukemia. A typical result of the blood changes in rats during the development of the transplanted lymphosarcoma is shown in Fig. 3 and in Table I.

The tumors resulting from the subcutaneous inoculation of leukemia cells grow to be very large, often attaining a size of 6×3 cm. by the 3rd week (Fig. 4). They differ from the usual transplanted tumor of the rat in that they actively invade the skin and under-

lying muscle (Fig. 5). Metastases to regional lymph nodes occur with some frequency. In 2 rats secondary growths were found in both eyes and another had a single eye involved. In these incidences the entire exposed part of the eyeball was covered by an opaque mass but at autopsy it was found not to involve either the eyeball or socket. The only point at attachment was by a thin pedicle to the inner canthus of the lids.

TABLE I: BLOOD CHANGES IN RATS DURING THE DEVELOPMENT OF TRANSPLANTED LYMPHOSARCOMA

	Inoculated intraperitoneally, developed leukemia	Inoculated intraperitoneally, no leukemia	Inoculated subcutaneously, local tumor
No. of rats	35	16	17
Days after inoculation	18	27	19
Average W. B. C.	76,100	9,280	10,600
Small lymphocytes	64%	54%	49.8%
Large lymphocytes	29.8%	13%	9.2%
Polymorphonuclears	6.0%	33%	40.9%

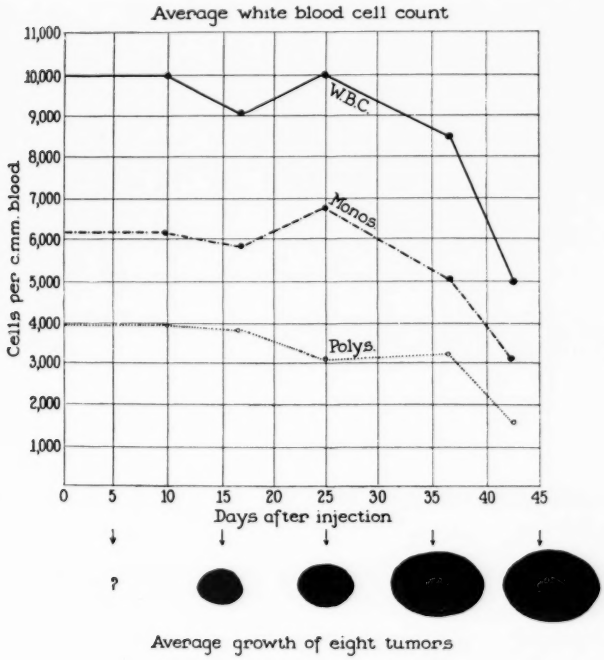

FIG. 3.—The white blood cell counts on 8 rats during the development of transplanted lymphosarcoma. The figures and measurements of tumors represent the average for the group.

The subcutaneous tumors are firm and even in the very large ones there is little evidence of central necrosis. Histologically they are composed of closely packed, small round cells of fairly uniform size with scant stroma. Many mitotic figures are seen, particularly in the young tumors.

At intervals the ability of the tumor cells to cause leukemia has been tested. Even after being carried as a local tumor for many generations, when the cells are inoculated intraperitoneally the rats rapidly develop fatal leukemia. There have been only two cases of intraperitoneal tumors without generalized leukemia.

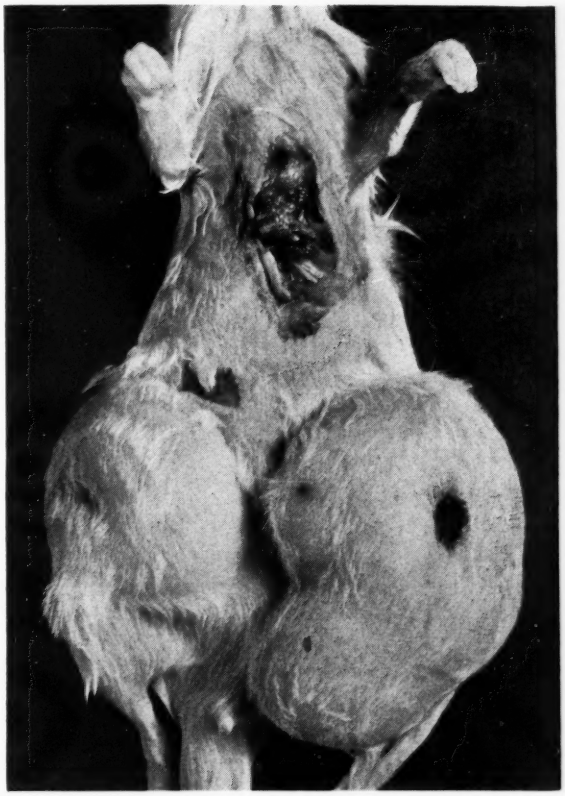

FIG. 4.—Local lymphosarcoma from subcutaneous inoculations of blood from a rat with leukemia.

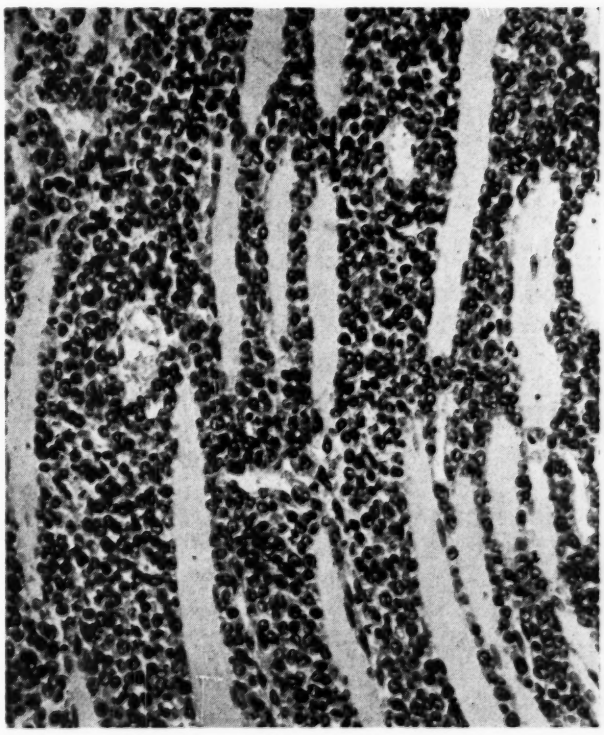

FIG. 5.—Extension of tumors shown in Fig. 4 into the muscles of the leg.

A summary of the transmission studies, with the number of animals utilized, is given in Fig. 6.

Results of inoculation of leukemic cells into the spleen.—The absence of any definite involvement of the spleen in the leukemia resulting from intraperitoneal inoculation in the presence of such general involvement has no evident explanation. It is generally considered that in the transmitted disease all of the leukemic cells are the descendants of those introduced by inoculation. To test the possibility that the spleen is relatively unsuitable for the development of the malignant cells, direct inoculations have been made into this organ.

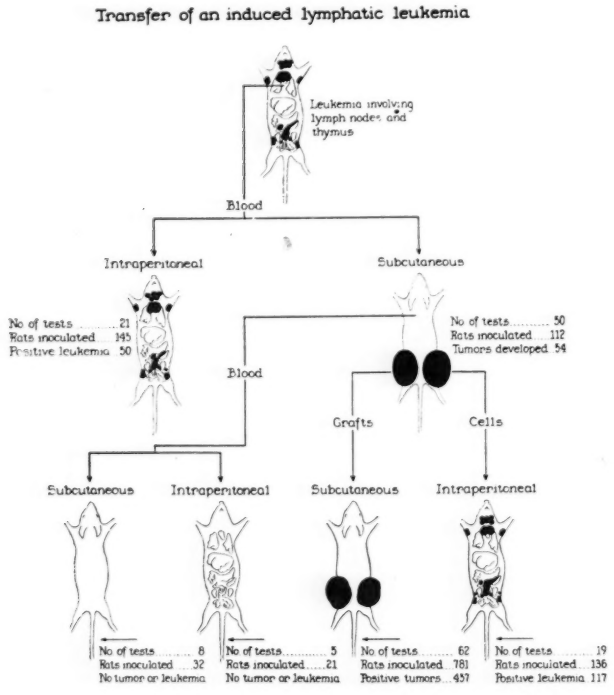

FIG. 6.—Transfer of an induced lymphatic leukemia.

Experiments.—The approximate size of spleens in the rats to be used was determined by direct measurement after bringing the spleen out through a small opening in the abdominal wall. The spleen was replaced leaving only the tip in the wound. About 0.5 cc. of leukemic blood was injected very slowly through a fine needle direct into the spleen pulp. In order to avoid leakage after withdrawal of the needle a gauze compress was held over the puncture till bleeding had ceased and the hole firmly blocked by clot.

Of the 16 rats so inoculated in three experiments, 8 were dead of the disease by the 6th day and only 2 survived as long as 10 days. The average length of life was 7.2 days. The blood showed a rapid rise in white cells, the average for the group being 60,000 per cu. mm., with 88 per cent lymphocytes. The spleens increased from 2 to 4 times their original size and in several instances were over 5 cm. in length. The livers were enlarged, markedly congested, and showed extensive round cell infiltration. The mesenteric lymph nodes were involved but there was no increase in size of any of the other nodes and no evidence of involvement of the thymus in any of the animals.

With the great number of leukemic cells in the blood, a wide dissemination through the organs and tissues would be expected. Yet the development of the secondary deposits is strictly limited depending on the site of inoculation. The principal involvements following intraperitoneal injection are the superficial lymph nodes and the thymus; splenic inoculation results in deposits principally in the spleen and liver; and subcutaneous inoculation gives local tumors with only regional lymph nodes affected.

Survival of cells in frozen tissue.—It (5) has been shown that several mammalian transplanted tumors may be preserved in a frozen state over long periods. In the earlier passages three attempts were made to transmit the rat leukemia by blood and involved lymph nodes which had been kept in a frozen state for a period, but no results were obtained. However, in later passages tissue kept frozen for 14 days and then inoculated subcutaneously produced tumors in all of the animals. The tumors were somewhat slower in starting but grew vigorously later. A spleen kept frozen for 21 days produced leukemia in all of the rats into which it was inoculated. Frozen blood has continued to give negative results.

Attempted transmission by cell-free material.—There have been no well authenticated examples of transmission of mammalian leukemia by cell-free material but for completeness attempts have been made with the present strain.

Experiments.—In five tests, the tumors or involved lymph nodes were frozen and desiccated *in vacuo*. The powdered desiccates taken up in water were inoculated into 69 rats. None of these developed either tumors or leukemia.

The disease is readily transmitted by the ascitic fluid from leukemic rats. This fluid collected from a number of such animals was filtered through paper and centrifuged sufficiently to bring down the cells. The supernatant fluid injected into 10 rats failed to induce the disease. The resuspended sediment containing cells produced leukemia in 6 out of 10 animals injected.

The possible presence of an agent in the serum of rats with leukemia has also been investigated. In two tests, 9 normal animals were inoculated with the serum and 11 with the sedimented blood cells from rats with advanced leukemia. None of those with the serum showed any reaction while 7 injected with the blood cells developed the disease.

Failure of transmission to other species.—Some 60 mice have been inoculated with blood from rats with leukemia either subcutaneously or intraperitoneally

but none of them showed any evidence of tumors or leukemia.

Using Green's method (5), rat leukemic cells were inoculated into the anterior chamber of the eye of 4 rabbits and 8 guinea pigs. There was no growth observed at any time and no surviving cells could be found at the end of 4 months.

Grafts of the rat lymphosarcoma were inoculated into the right frontal lobe of the brain of 12 mice and 2 rabbits (11). No evidence of growth or survival of the cells could be found in any of the animals. A number of other attempts have been made to induce leukemia in rats with carcinogenic chemicals but so far this has succeeded only in the one instance reported above. Further attempts are in progress.

Discussion

The close relationship between leukemia and malignant tumors is so generally conceded that it needs no further emphasis. There are now a number of examples of leukemias induced in mice by the same chemicals which are known to induce both carcinoma and sarcoma. In certain strains of mice the type of response to the carcinogenic chemical is influenced by the genetic tendency of the strain to develop certain types of spontaneous tumors. For example, Lynch and others have shown that the development of induced lung tumors in any given strain varies directly with the degree of normal tendency of the various strains to develop spontaneous lung tumors. Apparently the same principle holds for leukemia in mice as in most of the strains in which leukemia has been induced, the disease also is known to occur spontaneously. It is suggested that with limitations the carcinogenic agents accentuate the tendency to develop certain types of tumor or leukemia.

A point of interest in the present investigation is that leukemia has been induced in a species in which spontaneous leukemia is probably of very rare occurrence as no cases are on record. Lymphoid tumors have been observed but even these are rare in the strain of rats utilized for these studies according to the only available information.

There is a clear-cut variation in the manifestation of the transplanted disease, dependent on the site of inoculation. Why the spleen and liver should escape involvement under one condition and the lymph nodes and thymus escape under another, when the blood stream is teeming with the malignant cells, is not immediately explainable. Nor is it evident why the lymphosarcoma resulting from subcutaneous inoculation which actively invades all the surrounding tissues including metastasis to regional lymph nodes so rarely gives rise to generalized leukemia.

This first easily transferrable typical leukemia in a larger mammal gives new material for investigation of a type of malignant disease.

Summary

A rat which had been injected in both groins with 1,2,5,6-dibenzanthracene in benzol developed typical lymphatic leukemia. The first lesions were noted in the regional lymph nodes where crystals of the agent were found embedded in the tissue. It has proved possible to transfer the disease to other rats but the manifestations of the disease are varied depending on the site of injection of leukemia cells. When given intraperitoneally, the disease is manifested in the blood stream with involvement of only the lymph nodes and thymus. Subcutaneous inoculations with either blood cells or involved nodes with few exceptions produced local lymphosarcomas spreading only as far as the regional lymph nodes. Inoculation into the spleen produces leukemia with the principal manifestation in the spleen and liver leaving the superficial nodes and thymus untouched.

The transmitted disease is described.

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A Transmissible Lymphoid Tumor of the Chicken*

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The first demonstrations of the transmission of neoplastic disease in the chicken were made by Ellermann and Bang (3) with fowl leukosis and Fujinami and Inamoto (8) and Rous (25) with sarcomas. Subsequently, many spontaneous neoplasms of connective tissue of the chicken have been shown to be experimentally transmissible and in nearly all transmissible neoplasms a cell-free agent has been shown to be the cause. Many of the tumors induced experimentally in the chicken by the use of tar or certain of its derivatives have been found to be transmissible and from some of these tumors (17, 18) a cell-free agent capable of producing the neoplasms has been isolated. Connective tissue neoplasms are relatively infrequent as spontaneous tumors of the chicken. The more common type of spontaneous neoplastic disease of the chicken is composed of lymphoid elements and has been described by such designations as lymphocytoma, lymphomatosis, lymphosarcoma, leukosis, "big liver disease," and others.

The great economic loss to the poultry industry caused by lymphoid neoplastic disease has stimulated much research work. In spite of considerable investigation much confusion exists as to the transmissibility of lymphoid tumors and the relation of lymphoid tumors of the chicken to fowl paralysis. Some investigators, Andersen and Bang (1), Mathews and Walkey (19), Engelbreth-Holm (4, 5, 6), and Fenstermacher (7) report that they have been unable to transmit lymphocytoma from affected chickens to healthy chickens. Some workers present data to indicate that lymphocytoma and fowl paralysis have a common etiological agent, Pappenheimer, Dunn, and Seidlin (23), Seagar (26), Jungherr (15), and Gibbs (12). Others, Patterson, Wilcke, Murray, and Henderson (24), Johnson (14), and Lee, Wilcke, Murray, and Henderson (16), believe that fowl leukosis, fowl paralysis, and lymphocytoma are all caused by a single agent. An interesting transmissible disease of the lymphoid cell system of the chicken has been described by Furth (9, 10, 11). He has called this disease lymphomatosis, but believes it different from the common type of spontaneous lymphomatosis which is associated with marked enlargement of the liver ("big liver disease," "hepatolymphomatosis"). The transmissible lymphomatosis of Furth is caused by a filter-passing substance that he calls the strain 2 agent. The agent of strain 2 produced myelomatosis and endothelioma as well as lymphomatosis and erythroblastic leukosis. Some other strains of the fowl leukosis agent have the ability to produce sarcoma and most of the strains become somewhat modified in their disease-producing power with successive serial passage, Olson (20).

* Contribution No. 402 of the Massachusetts Agricultural Experiment Station.

This report describes a transmissible lymphoid neoplasm that has been developed and studied in experimental chickens during the past 3 years. The flock in which this tumor originated has been described (22).

Description of case 452 (transmissible lymphoid tumor).—The chicken was a crossbred, resulting from the mating of a Rhode Island Red female with a Barred Plymouth Rock male, purchased from a commercial hatchery and raised under the ordinary conditions for a small poultry flock. It first attracted attention at the age of 118 days when a small erosion of the skin on one toe was noted. A slow, persistent hemorrhage came from the lesion which healed without treatment. The bird was relatively light in weight. By October 30, 1937, at the age of 146 days, the chicken had become more emaciated and was brought to the laboratory. Its temperature was approximately normal (107.3° F.)

Numerous examinations of the blood were made during the illness of the bird. The results of the blood counts are given in Table I. Only differential blood counts were made before the chicken became ill and the counts were found to be within the range of normal variation. A résumé of the studies on the blood follows. The number of erythrocytes and the amount of hemoglobin in the blood decreased slightly during the illness of the chicken. No pathological forms of erythrocytes were observed. The thrombocytes were normal. The number of eosinophils and basophils varied slightly around the normal values for these cells. The total number of leukocytes was greater than normal at the time the chicken was first ill. This marked increase persisted for 10 days and then the number dropped to a more nearly normal value. The number of heterophils (pseudoeosinophils) was originally very high, then dropped, later increased, and finally fell to a nearly normal level. Lymphocyte numbers were normal at first, increased markedly during the early part of the illness, and near the terminal stage of the disease were again at a normal level. The number of monocytes was higher than normal at all stages of the disease and their total value was in general parallel to the number of heterophils. No immature cells were noted in the blood smear preparations, with the possible exception of the group designated as

monocytes. Included in the classification of monocytes were a few cells somewhat atypical yet possessed of certain characteristics of the monocyte. These atypical cells were considered to represent immature monocytes. In no instance did they constitute the bulk of cells designated as monocytes, but were rather relatively scarce.

Twelve days preceding death the chicken became so weak that it was unable to stand. From this time it was fed a mixture of ground feed and water by means of a bulb syringe. The chicken died November 24, 1937 at the age of 205 days. Necropsy was done immediately after death and was conducted in an aseptic manner, so that material for the inoculation of experimental chickens could be obtained reasonably free of contamination.

TABLE I: VALUES FOR BLOOD CELLS AND HEMOGLOBIN, CASE 452, LYMPHOID TUMOR

Date	Erythrocytes *	Hemoglobin †	Thrombocytes	Leukocytes: Total	Lymphocytes	Heterophils	Eosinophils	Basophils	Monocytes
					76.5	*13.5*	*4.0*	*1.5*	*4.5*
8/26/37	....	....	....	....	....	....	....	....	....
					77.0	*18.0*	*2.0*	*1.0*	*2.0*
9/2/37	....	....	....	....	....	....	....	....	....
					77.0	*16.0*	*1.0*	*2.0*	*4.0*
9/9/37	....	....	....	....	....	....	....	....	....
					81.0	*11.0*	*2.0*	*2.0*	*4.0*
9/16/37	....	....	....	....	....	....	....	....	....
					21.7	*60.0*	*0.5*	*0.8*	*17.0*
10/30/37 ‡	2.52	8.35	47,000	46,950	10,190	28,180	235	375	7,970
					35.8	*41.5*	*0.5*	*1.5*	*20.7*
11/1/37	2.46	8.03	41,000	60,305	21,600	25,000	301	904	12,500
					56.8	*23.6*	*0.8*	*1.0*	*17.8*
11/2/37	2.32	7.88	36,750	48,747	27,700	11,500	390	487	8,670
					71.5	*14.8*	*0.7*	*1.0*	*12.0*
11/3/37	2.47	7.50	28,900	50,214	35,900	7,440	352	502	6,020
					58.0	*26.0*	*0.7*	*1.0*	*14.3*
11/5/37	2.50	8.32	36,400	70,823	41,100	18,400	495	708	10,120
					50.0	*34.5*	*0.5*	*0.7*	*14.3*
11/6/37	2.44	8.09	43,500	55,363	27,700	19,100	276	387	7,900
					52.7	*35.8*	*0.7*	*0.8*	*10.0*
11/8/37	2.51	7.58	43,300	55,148	29,100	19,700	387	441	5,520
					69.0	*20.5*	*0.7*	*1.5*	*8.3*
11/12/37	2.32	7.58	30,200	26,288	18,150	5,380	184	394	2,180
					49.0	*27.8*	*0.2*	*3.0*	*20.0*
11/19/37	2.58	9.70	38,800	26,207	12,850	7,280	52	785	5,240

* Expressed in millions per cu. mm.
† Expressed in gm. per 100 cc. of blood.
‡ Values are averages of two or three counts and are number of cells per cu. mm. of blood.
Figures in italics are per cent values.

The carcass was markedly emaciated. The liver was moderately enlarged and contained a number of gray-white tumor masses. These tumor masses varied in size from a few millimeters to 2.5 cm. in diameter. They were discrete although some masses tended to be confluent. The spleen was of normal size and color and contained 4 small foci similar to those seen in the liver. Immediately dorsal to the cloaca was a solid rounded mass about 4.5 cm. in diameter composed of soft gray tissue. On one side of the large mass was a nodular protuberance which had a hollow center filled with dry, yellow, necrotic material which probably represented the lumen of the bursa of Fabricius. The larger solid tumor mass apparently arose from the wall of the bursa. The kidneys were dark red in color and small in size. The ovary was quiescent and normal in appearance. The marrow of the distal end of the left femur was replaced by a mass of gray tumor tissue; that of the right femur was red-brown in color and hyperplastic.

Microscopically the tumor was composed of large, round lymphoid cells. These cells had a relatively

large vesicular nucleus and their basophilic cytoplasm was confined to a narrow rim at one edge of the cell. Mitotic figures were numerous. The neoplastic lymphoid cells were extravascular in location, although in some organs, as the liver, some such cells were found intermingled with the elements of the blood within the vessels. The tumor masses in the liver were diffuse and tended to compress the liver tissue rather than to infiltrate it (Fig. 1). Tumor cells were actively multiplying in many of the periportal areas of the liver. Several small microscopic foci of neoplastic lymphoid cells were found in the spleen and there was also a rather generalized hyperplasia of the reticulum of the splenic pulp. An accumulation of

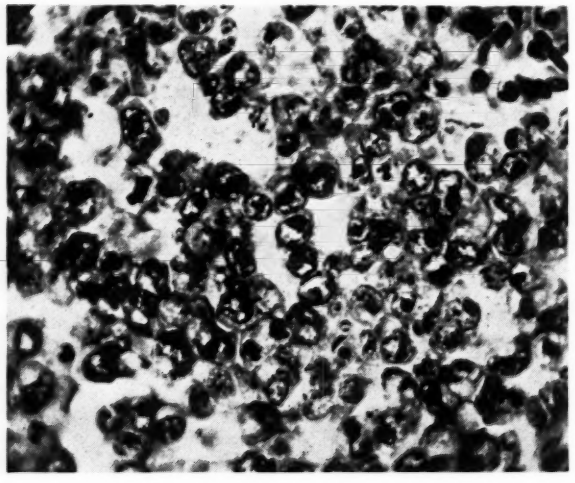


FIG. 1.—Section of tumor nodule in liver of original case of lymphoid tumor (case 452). The tumor cells have relatively large vesicular nuclei and distinct nucleoli. Magnification × 710.

the tumor cells on the visceral aspect of the splenic capsule penetrated the latter at some points and encroached upon the substance of the organ. The tumor in the wall of the bursa of Fabricius and in the bone marrow was composed of lymphoid cells. The myeloid tissue, where not replaced by tumor cells, was active, especially in granulopoiesis, and a normal relationship existed between erythropoiesis and granulopoiesis. The ovary was infiltrated with tumor cells. The kidneys, lungs, lumbar spinal cord, spinal cord in the region of the brachial plexus, and adjacent dorsal root ganglia were normal in microscopic appearance.

TRANSMISSION OF THE LYMPHOID TUMOR

Methods.—In the first passage of the lymphoid tumor the trocar and cannula method of implanting tissue into the recipients was used. The usual precautions to prevent contamination were observed in obtaining the tissue, during the process of mincing with scissors, and while implanting tissue into the experimental chicken. This technic was found to be quite laborious, particularly when large numbers of birds were used. An instrument, called a tissue mincer (21), was devised which subdivided the tumor tissue to such a size that it could be injected with a syringe using a needle with a large bore (15 gauge). Handling of the minced tumor was facilitated by dilution of the tissue with an equal volume of modified Ringer's solution (13). The standard dose of such a mixture was 0.5 cc. The site of implantation of the inoculum was usually the breast muscle on one or both sides. In instances where several sites were used, inoculations were made into the musculature of the thighs, as well as into the breast muscle. The implants were placed deep in the muscle tissue and in some experiments in the subcutaneous tissue as well.

Source of experimental chickens.—The chickens used in the first 30 passages of the lymphoid tumor were obtained from three sources, all unrelated to the flock contributing the bird from which the tumor originated. The principal source of supply was a small flock of laying hens maintained in the laboratory for the purpose of producing inbred chickens for experimental work. These Rhode Island Red hens were sisters and were mated to a brother. The other two sources of birds were another breeding stock of the laboratory and stock from a commercial hatchery.

First passage of the lymphoid tumor.—The first attempt at transmission of the disease found in case 452 was made at the time the bird was brought to the laboratory, October 30, 1937. Blood was taken from the wing vein in sodium citrate solution and inoculated intravenously in 1 cc. doses into 4 experimental chickens 46 days old. These chickens remained normal and the experiment was terminated 73 days after inoculation.

At the time of necropsy of case 452, blood from the heart chambers and liver tissue infiltrated with tumor was obtained for the purpose of inoculating chickens.

A portion of liver infiltrated with lymphoid tumor was cut into small pieces with scissors and implanted in chickens with a trocar and cannula. Four chickens 13 days old received the inoculum in the peritoneal cavity. One died of an unrelated disease 30 days after inoculation and the others were killed 93 days after inoculation. The implants did not develop or produce disease in any of the chicks.

Six chickens 121 days old received implants of the diseased liver in the pectoral muscles of one breast. No reaction was noted at the site of implantation of lymphoid tumor tissue in 4 chickens and 48 days after inoculation they were considered negative and used for other purposes. One of the birds considered negative (M 34) was killed 139 days after inoculation

and found at necropsy to have a markedly enlarged liver infiltrated with lymphocytoma associated with foci of the tumor in the spleen and kidneys. This case of lymphocytoma has been considered spontaneous in origin. Two other birds of the group were killed 139 days after inoculation and were negative. Two of the chickens (M 31 and M 35) had detectable swelling at the site of implant 9 days after inoculation. This swelling continued to increase in size in each bird until at the time of necropsy the major portion of the pectoral muscles was replaced by a huge mass of neoplastic tissue. A biopsy of M 35 was made 10 days after inoculation to obtain tissue from the tumor in the breast muscle for study and implantation in other experimental chickens. The tissue had a marked infiltration with large, actively multiplying lymphoid cells between atrophic muscle fibers. Chicken M 35 died 49 days after it had been inoculated. The tumor mass in the right breast muscle measured roughly 15 x 7 x 6 cm. and was relatively firm and composed of gray tissue (Fig. 2). A discrete, disc-shaped mass of tumor, 23 mm. in diameter and 11 mm. thick, was found on the surface of the left liver lobe. Except for this, the liver and the rest of the visceral organs were normal. Histologically, the breast tumor was composed of large, neoplastic, lymphoid cells having the appearance of those described in case 452, from which the tumor was derived. A microscopic, metastatic focus was found in the spleen and a small embolus of tumor cells was noted in a blood vessel of the liver. Repeated examinations of the blood of M 35 were made during the course of the experiment. Sixteen days after the implant of the lymphoid tumor, a moderate leukocytosis of heterophils was noted, but this did not occur again. Chicken M 31 was killed 51 days after inoculation with the lymphoid tumor to provide material for inoculation of other chickens. No gross pathological change was noted except for the large tumor mass of the pectoral muscle. Upon histological examination several small, discrete metastatic foci of tumor cells were found in the spleen. Repeated examinations of the blood of the bird during the period of experiment were made and no pathological changes were noted.

The blood obtained from the heart chambers of case 452 at the time of necropsy was kept from clotting by the addition of sodium citrate solution and was injected into the right wing veins of 4 chickens aged 13 days. The blood of these birds was examined periodically after their inoculation, but no abnormality was observed. Three of the chickens were negative at the time the experiment was terminated 96 days after inoculation. The remaining bird, M 78, was noted to hold its right wing in an unnatural position 82 days after inoculation. Upon closer examination a large irregularly shaped tumor mass was found in the region of the right humerus (Fig. 3). A biopsy was made and the tumor was found to consist of large, neoplastic lymphoid cells. Another biopsy was attempted, 99 days after inoculation, during which the bird died. The tumor of the wing measured approximately 7 x 6 x 5 cm. and it completely encircled the humerus by infiltration of the soft structures. It was yellow-gray in color and well vascularized. Portions of the tumor were used for inoculation of other experimental chickens and it was through this bird that serial passage of the lymphoid tumor was maintained.

Second passage of the lymphoid tumor.—The tumor material secured from chicken M 35 at biopsy 10 days after inoculation was implanted in the peritoneal cavity of 4 chickens aged 16 days. One died 4 days later and was negative, except for a localized peritonitis. The other 3 remained negative and the experiment was terminated 83 days after inoculation. The tumor was also inoculated with a trocar and cannula into the pectoral muscles of 4 chickens aged from 218 to 235 days. In only one bird did the implant develop. It attained a maximum size at the end of 20 days, and then regressed. All 4 birds were negative when killed 83 days after inoculation.

A portion of the tumor from chicken M 31 was implanted in the breast muscle of 4 chickens, 57 days old. All the birds remained negative and were killed 42 days after inoculation. Another portion of the tumor from M 31 was ground into a heavy emulsion with a modified Ringer's solution (13) and was injected in 0.5 cc. doses into the breast muscles of 6 chickens aged from 43 to 57 days. No indication of growth of implant was observed and the experiment was concluded after 42 days.

Intramuscular and subcutaneous implants of the tumor from chicken M 78 grew in all of the 6 birds so inoculated. In four instances the growth of the tumor reached a maximum size about 14 days after inoculation and then regressed. One bird died at 28 days with a large tumor at the site of inoculation and infiltrative metastases in the ovary, adrenals, and proventriculus. The remaining chicken had a large tumor of the breast muscle when it was killed 28 days after inoculation to provide material for further passage of the neoplasm.

Third to thirtieth passages of the lymphoid tumor.—During the course of the subsequent serial passages various factors such as age of the chickens at time of inoculation, intervals between passages, and mode of inoculation were varied to study their effect on the results obtained.

Pathology

A total of 442 chickens was inoculated in the intramuscular or subcutaneous tissues with the lymphoid tumor, during its first 30 serial passages. Growth of the implant was observed in 300 birds or 67.7 per cent of those inoculated (Table II). After the growth of the implant had become established in some animals the tumor regressed, in others it continued to grow but remained localized, and in still others it grew and metastasized to visceral organs.

Regression of the tumor growth was observed in 133 birds or 44.3 per cent of the chickens in which the implants took root and developed. Growth of an implant was usually apparent about 7 days after inoculation, and in a few instances it began to regress shortly after it could be detected. On the average the maximum growth was observed in these birds 13.6 days after inoculation. In one bird the tumor attained its maximum growth 34 days after implantation and then completely receded. Most of the chickens in which the implant of tumor regressed were killed on an average of 40.8 days after inoculation. The remainder of the birds (6 birds only) died from unrelated causes from 17 to 74 days (average 54 days) following inoculation.

The lymphoid tumor remained localized at the site of inoculation in 116 or 38.7 per cent of the birds in which growth took place. In this group 48 died and 68 were killed. Of the birds killed many were *in extremis,* others were killed to furnish material for transmission experiments, and still others to end the experiment of which they were a part. The duration of life during the experiment was 11 days in the shortest instance and 66 days in the longest. The average duration for birds that were killed was 22.5 days and for birds that died was 24.7 days. In many cases the tumor grew to a large size and weighed approximately one-fifth the total body weight, which with some exceptions, seemed to be the limit of growth (Fig. 4).

Metastases from the tumor were observed in 51 chickens (17 per cent of all birds in which growth was observed). In 21 birds one organ contained metastases; in 12 birds two organs were involved; in 8 birds three organs; in 5 birds four organs; and in 4 birds six organs. One bird used in the 28th passage of the tumor showed at necropsy 16 days after inoculation diffuse infiltration of neoplastic lymphoid cells in the liver, kidneys, adrenals, proventriculus, bone marrow, and thymus in addition to the breast muscle at site of implant. Instances of this type of diffuse metastasis have been frequently observed in more

Table II: Summary of Thirty Serial Passages of a Transmissible Lymphoid Tumor (Intramuscular or Subcutaneous Implants)

Passages	Number inoculated	Negative	Growth	Result of growth: Regression	Result of growth: Local only	Result of growth: Local and metastases
		31.6	*68.4*	*37.8*	*48.8*	*13.4*
1 to 10	120	38	82	31	40	11
		33.7	*66.3*	*37.2*	*37.2*	*25.6*
11 to 20	154	52	102	38	38	26
		31.4	*68.6*	*55.2*	*32.8*	*12.0*
21 to 30	169	53	116	64	38	14
		32.3	*67.7*	*44.3*	*38.7*	*17.0*
Total	443	143	300	133	116	51

Figures in italics are per cent values.

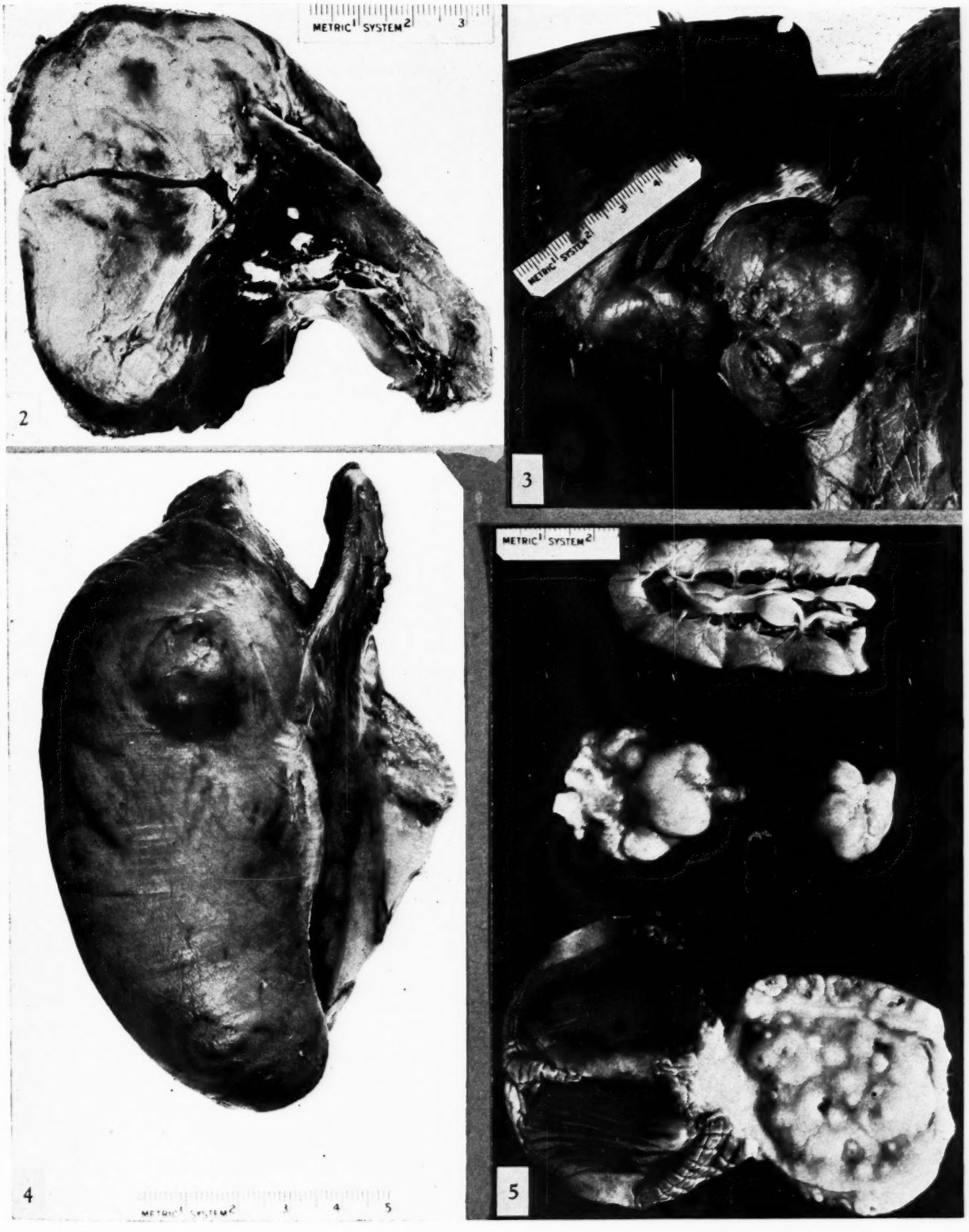
DESCRIPTION OF FIGURES 2 TO 5

Fig. 2.—Cross section of sternum and both breast muscles of chicken M 35. The musculature of the right breast is largely replaced by growth of lymphoid tumor implanted 45 days previous to necropsy. The line of separation represents the division caused by the sheaths of the deep and superficial pectoral muscles.

Fig. 3.—Tumor mass of wing of chicken M 78. This mass developed after a supposedly intravenous inoculation of blood from the original chicken with lymphoid tumor. Photograph taken 82 days after inoculation.

Fig. 4.—Ventral view of sternum showing the large tumor mass infiltrating the right breast muscle. The tumor developed in 45 days from an implant of the 20th serial passage. The mass was estimated to weigh 200 gm. (actual weight of entire specimen with sternum, 244 gm.) which represented nearly ⅓ the total carcass weight of 665 gm. at necropsy.

Fig. 5.—Pancreas and duodenum, heart, adrenal glands, and gizzard and proventriculus of a chicken which died 21 days after receiving an implant of the lymphoid tumor. Note the nodular metastatic foci in the pancreas and heart and the diffuse metastases in the wall of the proventriculus. Both kidneys of this bird likewise contained diffuse metastases.



FIGS. 2–5

recent experiments with the transmissible lymphoid tumor. Such cases usually die 9 to 12 days after inoculation. This case resembles the diffuse lymphocytoma so commonly observed in routine necropsy work of the veterinary diagnostic laboratory.

The heart and proventriculus were sites of predilection for metastases, which were observed 24 times in each organ. The adrenals were involved in 21 cases. Foci of tumor were found in the substance of the liver in 7 cases, and in 13 instances a mass of neoplastic lymphoid tissue was found in the region of the gall bladder fossa. Other organs found involved with metastases and the number of instances of involvement were as follows: kidneys, 12; gonads, 9; spleen, 6; thymus, 4; bone marrow, 3; mesentery, 3; lung, 2; and thyroid, 1. In the heart, liver, and kidney the metastatic foci were usually circumscribed and easily detected upon gross examination. Those in the spleen were usually much smaller and often not apparent until the organ was examined histologically. Diffuse infiltration of neoplastic lymphoid cells was characteristic in the wall of the proventriculus, adrenals, thymus, gonads, bone marrow, and mesentery (Fig. 5). Of the 51 birds with metastases 31 died between 15 and 49 days after inoculation (average 27.7 days) and 20 were killed between 14 and 52 days after inoculation (average 25.4 days). The earliest time at which focal metastasis was observed was 14 days after implant of the inoculum (noted in 2 cases). In one of these, microscopic foci only were found in the spleen, and in the other, tumor foci in the liver and kidney were apparent to the unaided eye.

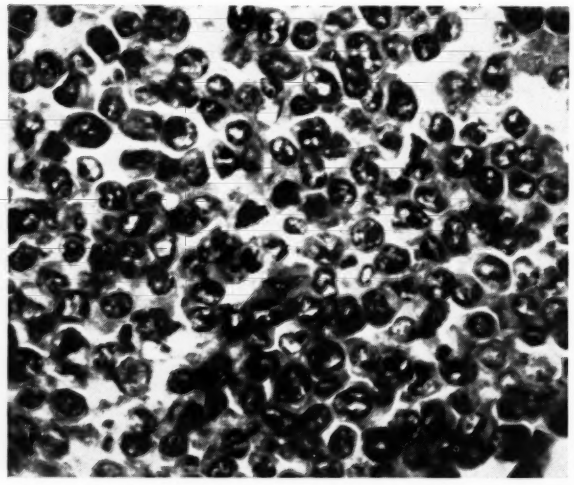


FIG. 6.—Section of tumor developed at site of implant in 10th serial passage. The character of the tumor is the same as illustrated in Fig. 1. Magnification × 710.

The transmissible lymphoid tumor retained its general cell type characteristics throughout the 30 serial passages (Fig. 6). The cell was somewhat larger than the young lymphoid cells in the normal lymphoid tissues. The nucleus, in tissue section preparations, was vesicular with chromatin arranged in clumps and condensed at the nuclear membrane. A relatively large and distinct nucleolus was usually evident. The cytoplasm was deeply basophilic with routine hematoxylin-eosin stain and relatively scant, being usually confined to one side of the cell. Imprint preparations stained with the May-Grünwald and Giemsa combination blood cell stain showed the nucleus to have a very fine and distinctive arrangement of the chromatin and parachromatin, not unlike the arrangement observed in the very immature lymphoid cells of transmissible fowl leukosis. One or two nucleoli were faintly apparent with the blood stain. The cytoplasm was finely granular and colored blue. In some cells a few azure granules were noted in the cytoplasm. There was a marked degree of uniformity in size and appearance of the tumor cells. The method of growth was usually infiltrative although in some organs in which the metastases were focal, as in the spleen, liver, and kidney, a more or less well defined capsule restricted the tumor. In many instances when the implant had been growing for a week, a variable amount of dry necrosis was found in the substance of the tumor. This was usually scattered in small areas. Frequently degenerated tumor cells with pyknotic nuclei were observed in sections. Fibrosis and scar tissue was all that remained at the site of implantation in those chickens in which growth had taken place and later regressed. The histological section of such an area made shortly after regression revealed many small foci of tumor cells circumscribed by fibroblasts (Fig. 7). Loose connective tissue containing many macrophages occupied the area formerly taken up with tumor. In birds killed some time after complete regression only a slight amount of connective tissue remained. No alteration was found at the site of inoculation in those birds in which the implants failed to grow.

Other features noted in chickens in which the tumor had been present for two or more weeks preceding death were emaciation, atrophy of the thymus, and hyperplasia of the myeloid elements of the bone marrow, particularly those involved in granulopoiesis.

DISCUSSION

For the present, only such factors as appear well substantiated by the results of the first 30 passages of the lymphoid tumor will be discussed.

During the early passages of the tumor the period between transmission experiments was spread over a considerable time range (from 7 to 89 days). It was later found that reliable inoculums could be prepared from tumors resident in the host for 14 days and this

was the age of the tumor used in the later passages. The interval between passages was on the average longest in the first 10 passages (24.5 days), 19.9 days for passages 11 to 20, and shortest for the last 10 (average 15.6 days). Despite this variation the results were about the same in the three groups (Table II).

Intramuscular implantation of the inoculum usually gave rise to a larger and more active tumor growth than subcutaneous implantation. The reason for this may be a more adequate blood supply in the muscle than in the loose connective tissue.

The age of the chicken at the time of inoculation appeared to be a significant factor affecting the fate

FIG. 7.—Section at site of implant after regression of tumor growth. Slight but distinct growth of the implant was observed 15 days after inoculation. The bird was killed 3 days later and only slight fibrosis was observed in the area. In the field illustrated there are several foci of tumor cells, one in the substance of the muscle and the others are surrounded by fibrous connective tissue. Magnification × 60.

of the implant. Chickens in which the tumor grew and those in which it metastasized were on the average aged 57.9 and 58.7 days, respectively (Table III). Birds in which regression occurred after growth were on an average 101.6 days old at time of inoculation. Birds which remained negative had an average age of 89.9 days at the time of inoculation.

Sex of the host had no effect on the results. Chickens from three groups of inbred stock representing different matings and from two other sources were used, but no effect could be attributed to the genetic composition of the host.

Fowl paralysis developed in 15 of the 443 birds (3.38 per cent) inoculated with the tumor, and lymphocytoma was observed in 6 birds (1.35 per cent). First symptoms of fowl paralysis were observed from 1 to 58 days (average 21.3) after inoculation. Fowl paralysis was also observed in 13 of 151 uninoculated chickens (8.61 per cent) held as controls. The average age at which symptoms were first observed in inoculated birds was 72.86 days and in control birds 67.6 days. Two of the 6 cases of lymphocytoma found in inoculated chickens could be interpreted as representing metastasis of the implant. To regard them in this light would, however, imply ability of the tumor to metastasize within 5 days after inoculation in one case and an uninterrupted growth of a metastic focus after complete regression of the implant in the other case. For the present such interpretation does not seem justified. Three instances of lymphocytoma were noted among the 151 control chickens (1.98 per cent).

Numerous unsuccessful attempts have been made to transmit other spontaneous lymphoid tumors (lymphocytomas) of chickens by the methods which were used in this instance. The types of disease described as lymphomatosis and transmitted by Jungherr (15), Johnson (14), Patterson *et al.* (24), and Lee *et al.* (16) are diverse and associated with a much longer incubation period than was observed with this lymphoid tumor. The strain 2 agent of Furth (9, 10) also produced more than one type of disease in birds into which it was inoculated. The transmissible lymphoid tumor described in this report has maintained its general characteristics and has reproduced itself as essentially the same type of disease in a large number of experimental chickens.

TABLE III: AGE OF HOST AT TIME OF INOCULATION CORRELATED WITH RESULTS

Result	Age (in days) at inoculation		
	Average	Maximum	Minimum
Metastasis	58.7	134	22
Local growth only	57.9	141	1
Regression	101.6 *	764	19
Negative	89.9	764	5
Total	81.2	764	1

* Only 34 of the 133 chickens in this group were over 85 days old, which makes the average disproportionately high.

SUMMARY

A transmissible lymphoid tumor of the chicken is reported and the results of the first thirty serial passages of the tumor are described.[1] Transmission has been accomplished by transplants of tumor tissue. These transplants failed to grow in some chickens, in others regression took place after a short period of growth, in still others growth was progressive and in some instances metastasized to the visceral organs. The results gave no indication of an etiological con-

[1] Later reports will discuss blood changes and attempts to demonstrate transmissibility by a cell-free agent.

nection between the lymphoid tumor, fowl paralysis, or fowl leukosis. The transmissible neoplasm is unlike any other transmissible tumor described in the chicken.[2]

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[2] Since this was written an abstract of an article (Transplantable Lymphosarcoma of the Chicken, Cancer Research, **1**:69-70. 1941) by Pentimalli has appeared, in which he described a transplantable lymphoid tumor. The original case from which the tumor was derived and the behavior of the tumor in experimental chickens was very similar to that observed in the present work.

The Experimental Production of Fibrosarcomas of Bone*

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From a desire to obtain experimental material for a continuation of studies on phosphatase in bone tumors (5, 6) an attempt has been made for over four years to produce sarcomas of bone in rats and mice.

The spontaneous occurrence of bone tumors in animals is so rare that it is a very uncertain source of experimental material. Pybus and Miller (12) have succeeded, however, in developing a strain of mice with a high incidence of spontaneous bone tumors which may prove to be of value in future experimental studies.

Martland's observation (11) on the development of osteogenic sarcomas in radium dial painters stimulated many attempts to produce experimental bone tumors with radioactive substances. Production of bone tumors has subsequently been reported by Sabin, Doan, and Forkner (15) to follow intravenous administration of radium and of mesothorium. Bone tumors were produced by implantation of radium sulfate, by Daels and Biltris (4); by intramedullary implantation of mesothorium, by Schürch and Uehlinger (16-18); and by subcutaneous injection of thorotrast, by Selbie (19). In all these instances the radiation from the radioactive material was unfiltered. However, bone tumors have also been reported by Lacassagne (8) after interstitial implantation of filtered radon, and were observed by Ross (13) to occur after the implantation of unfiltered radium.

Bone tumors in animals have also followed heavy application of filtered radium applied externally (7) and also following exposure to x-rays, as reported by Lüdin (9). A spindle cell sarcoma following x-ray treatment was experimentally produced in a rabbit as early as 1910 by Marie, Clunet, and Raulot-Lapointe (10). Bone tumors have therefore been found to result from prolonged exposure to both filtered and unfiltered rays from radium, unfiltered radiation from thorium, and roentgen radiation.

Brunschwig (2) has shown that transplantation of epidermoid carcinoma beneath the periosteum or into the bone marrow cavity produces spicules of new bone as in osteogenic sarcomas but, as Wephwadze (21) suggests, these spicules are probably formed by the osteogenic elements of the exfoliated periosteum and are not true tumors of bone.

The first tumor containing bone elements which was produced by carcinogenic chemicals was reported by Anardi (1) in 1934. He produced an osteochondroma in the ear of a rabbit by painting with tar. During the course of the present experiments, Brunschwig and Bissell (3) have reported the production of an ossifying sarcoma in a mouse following implantation of benzpyrene and cholesterol and a parosteal or central fibrosarcoma by intramedullary injection of methylcholanthrene. Sutro and Pomerantz, (20) by injecting 1,2,5,6-dibenzanthracene in benzene or lard in or near bones or joints, were able to produce cellular fibrosarcomas, some resembling rhabdomyosarcoma or myxosarcoma, and others somewhat resembling liposarcomas. No osteogenic sarcomas were produced, and although most of the tumors abutted on skeletal tissue, none showed new bone formation. Rusch, Baumann, and Maison (14) have reported the production of 3 periosteal fibrosarcomas in rats following injection of 3,4-benzpyrene in corn oil in two instances, and 1,2,5,6-dibenzanthracene in corn oil in the third, into the bone marrow.

Experimental

In the present experiments the upper end of the tibia in rats and mice was selected as the site of implantation of the carcinogenic agents because the bone at this site is large enough to be used conveniently in both species. At this point, also, the epiphyseal line can be exposed so that the agent will lie in contact with it. In view of the common occurrence of osteogenic sarcomas in relation to the epiphysis of the bone, this seemed the most likely site to invite the development of osteogenic types of bone tumors. A summary of the experiments is given in Table I.

A small incision was made over the antero-lateral surface of the upper end of one or both tibiae and a small drill hole made at the lower margin of the epiphyseal line aided by the magnification of a Beebe loupe. The small crystal or pellet of the substance used was inserted into the depths of the drill hole and a small fragment of muscle was packed into the hole to arrest hemorrhage and to hold the substance in position. The wound was closed with silk. In a few animals a small crystal of methylcholanthrene was placed against the bone on the fibular side of the upper third of the tibia after elevating the periosteum slightly.

It was found that our Slonaker strain of rats was extremely resistant to the agents used and no tumor developed. In an early series, young adult rats were used. The experiment was repeated in very young rats in order that the agent might act through the age period corresponding to puberty and adolescence in the human, when osteogenic sarcomas most frequently appear. A crystal of methylcholanthrene was therefore placed against the epiphysis of the tibia in

* This investigation was aided in part by a grant from the Committee on Research in Endocrinology, National Research Council.

14 rats less than 15 days old. Although the last animal died 635 days after implantation, no tumor had developed. In 2 rats in which a radon seed had been implanted, fractures occurred at the site in both animals about 2 months postoperatively. These fractures appeared to unite firmly again, but the animals died subsequently of intercurrent disease without tumors. Unfortunately, a disease which produced a large inflammatory mass about the cecum with accompanying diarrhea attacked the rat colony and many animals died prematurely from this cause.

tion of thorotrast. However, these crystals were readily soluble in the tissue fluids and the solution appeared to be transported quickly from the site of implantation. No tumors have developed although 11 of the 12 mice lived for more than 1 year after implantation. It is possible that if the removal of the thorotrast from the site of implantation is prevented by mixing with vaseline, as Schürch and Uehlinger (16-18) have done, the likelihood of the development of tumors at the site will be increased.

We have presumed that all these fibrosarcomas were

TABLE I: EXPERIMENTS ON BONE

Animals	No.	Substance used	Site	Tumors produced	First observation of palpable tumor
Rats	11	10% methylcholanthrene in cholesterol	Upper tibial epiphysis	None	
Rats	2	Cholesterol	Upper tibial epiphysis	None	
Rats	4	100% methylcholanthrene	Upper tibial epiphysis	None	
Rats (15 days old)	14	100% methylcholanthrene	Upper tibial epiphysis	None	
Rats	2	Radon seeds (0.16 mc. each)	Upper tibial epiphysis	None	
Rats	9	Thorotrast crystals	Upper tibial epiphysis	None	
Mice (C3H)	20	100% methylcholanthrene	Upper tibial epiphysis	16 fibrosarcomas in 13 animals	91 to 225 days
Mice (DBA)	2	100% methylcholanthrene	Upper tibial epiphysis	None	
Mice (A)	12	Thorotrast crystals	Upper tibial epiphysis	None	
Mice (C3H)	5	100% methylcholanthrene	Tibia subperiosteal	None	
Mice (A)	5	100% methylcholanthrene	Tibia subperiosteal	1 fibrosarcoma	132 days

Of the 13 mice which developed fibrosarcomas at the site of implantation in the upper tibial epiphysis, 3 developed bilateral tumors almost simultaneously. All the tumors were fibrosarcomas, usually of spindle cell type, but with some undifferentiation (Figs. 1 and 2). Two of these tumors are shown in Figs. 3 and 4. None, however, showed new bone formation except in immediate relation to the periosteum where, in the roentgenograms, small areas with radiating spicules of bone could be seen.

The application of methylcholanthrene against traumatized periosteum was no more successful in producing true bone sarcomas, as only 1 tumor was produced by this method, a spindle cell sarcoma without osteogenic characteristics.

Because of the reported successes in the production of osteogenic sarcoma by the use of radioactive substances, an attempt was made to produce similar tumors with the crystals resulting from the evapora-

of parosteal origin, for by following the process at the site of implantation, it was seen that a small zone of necrosis developed about the agent and was followed by repair by fibroblasts which appeared to enter the area from the extra-osseous connective tissue through the small drill hole in the bone. The development of malignant tumors subsequently occurred, probably from this repair tissue.

In three instances where methylcholanthrene had been implanted into both tibiae, tumors were discovered on both sides when microscopic sections were examined, although a tumor had been palpable on only one side (Figs. 3 and 4). This would seem to indicate that similar tissues in an individual mouse respond equally to similar stimuli and may develop tumors almost simultaneously.

It is interesting to note in this connection that most of the mice of the strain of Pybus and Miller (12) in which a high incidence of spontaneous bone tumors occurred had multiple tumors.

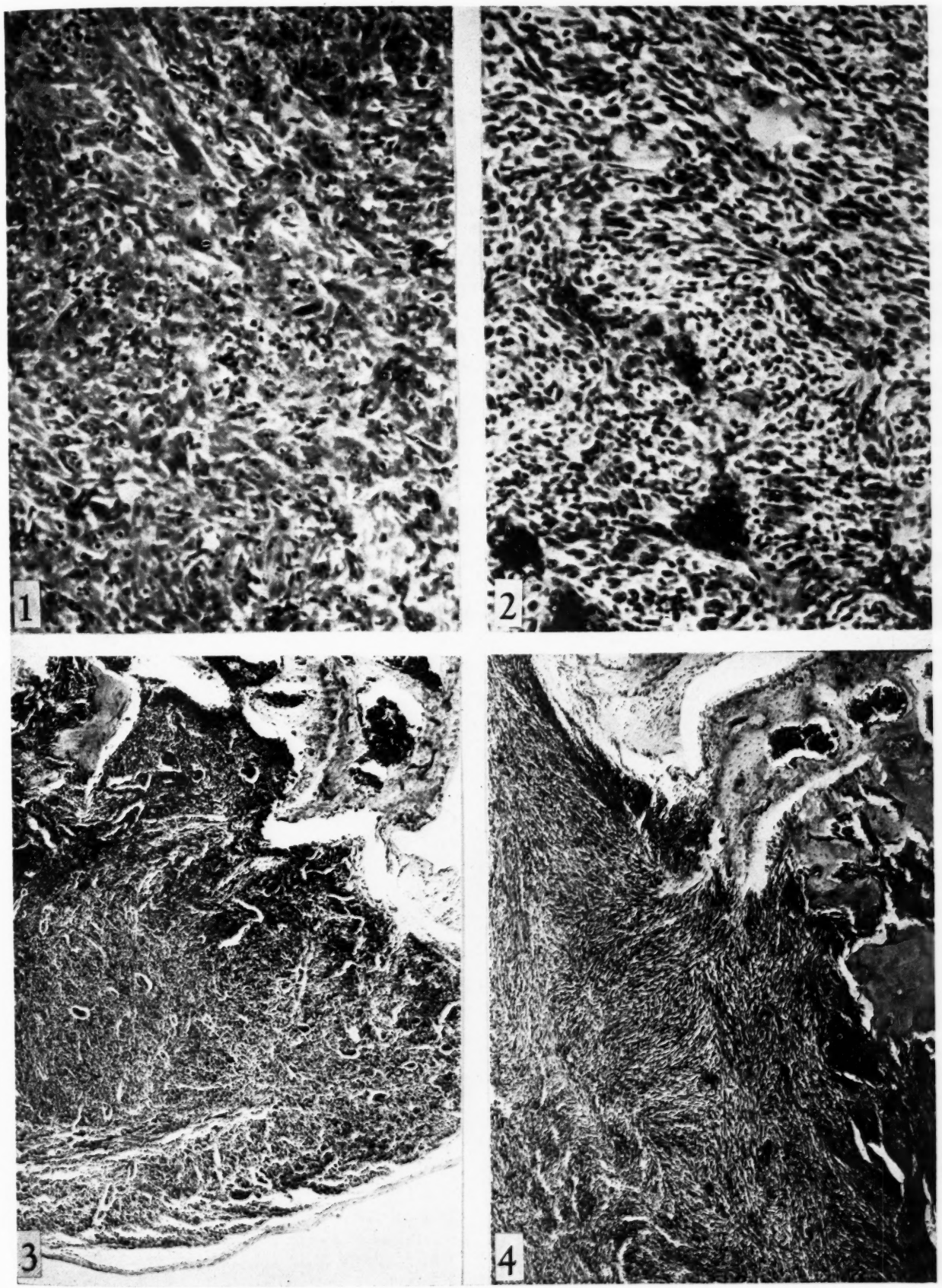
DESCRIPTION OF FIGURES 1 TO 4

FIG. 1.—Fibrosarcoma showing considerable undifferentiation appearing 211 days after introduction of methylcholanthrene into the head of the right tibia of a mouse of C3H strain.

FIG. 2.—Fibrosarcoma with giant cells which appeared 176 days after a crystal of methylcholanthrene had been placed in the head of the right tibia of a mouse of C3H strain.

FIG. 3.—Fibrosarcoma produced by methylcholanthrene in head of left tibia after 178 days, in a mouse of C3H strain. A similar tumor was found in the opposite leg. (See Fig. 4.)

FIG. 4.—Fibrosarcoma produced by methylcholanthrene in the head of the right tibia after 178 days, appearing simultaneously with the tumor in the opposite tibia shown in Fig. 3. The epiphyseal line may be seen in the right upper corner.



FIGS. 1-4

Brunschwig has suggested that fibrosarcomas develop from carcinogenic agents in bone because "the number of fibroblasts in bone which cannot function as osteoblasts is much in excess of active and potential osteoblasts and . . . . the carcinogenic agent therefore is in contact with relatively greater numbers of the former." However, it is possible that the more highly differentiated tissues are more resistant to the carcinogenic agents than are young fibroblasts which engage in the repair process. This repair process is so active that the agent quickly becomes surrounded by young fibroblasts and contact with the more differentiated cells cannot be maintained.

As many investigators have shown, when radium or mesothorium is injected intravenously or ingested, the deposition occurs preferentially in bone. In these cases the action of the rays from the radioactive substance must in many cases act upon fibroblasts as well as upon osteoblasts, yet the predominance of tumors of osteoblastic origin resulting from these substances is well known (11). An alternate hypothesis is, of course, that a certain selectivity exists so that the fibroblasts are more susceptible to the carcinogenic action of chemical agents and the osteoblasts more susceptible to the carcinogenic action of radioactive substances. In the experiments of Selbie (19), a thorotrast deposit lay in contact with a rib, presumably in contact with both fibroblasts and osteoblasts, and an osteosarcoma developed. In experiments of Schürch and Uehlinger (16-18) in which vaseline containing radium or mesothorium was inserted into the medullary cavity of the femur of rabbits, 4 osteogenic sarcomas and 1 Ewing's sarcoma were found among the 9 tumors which resulted. The remaining tumors were fibrosarcomas, suggesting in this case that, with the application of these substances in contact with both fibroblasts and osteoblasts, an approximately equal susceptibility to the development of malignancy exists.

Phosphatase determinations on 3 of the fibrosarcomas reported here showed only the faintest traces of phosphatase, a characteristic of sarcomas of fibroblastic origin in both animals and humans in contrast with a high phosphatase activity in the tissues of osteogenic sarcoma. This suggests that the tumors produced in these experiments were not true osteogenic sarcomas but were sarcomas of extra-osseous origin; since the experiments of Franseen and McLean (5) have shown that in tumors of osteoblastic origin, even when osteoid tissue is absent and a histological picture characteristic of fibrosarcoma obtains, phosphatase is produced in the tumor tissue.

Summary

Attempts have been made over a period of 4 years to produce osteogenic sarcomas in rats and mice by implanting methylcholanthrene, thorotrast crystals, and radon seeds near the upper tibial epiphysis and also methylcholanthrene subperiosteally. Seventeen tumors were produced in mice but all proved histologically to be fibrosarcomas of probable extra-osseous origin.

The authors are indebted to Dr. Shields Warren and Dr. Olive Gates for assistance in interpreting the microscopic sections of the tumors produced, and to Miss Kathryn Hooper for preparation of the photomicrographs.

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The Cancer Producing Properties of Azo Compounds in Mice

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Industrial cancer in its various forms has stimulated the researches which have led to the discovery of the cancer producing properties of the various carcinogens. Recognition of the high incidence of cancer of the scrotum of chimney sweeps led eventually to isolation from coal tar of 3,4 benzpyrene, the compound responsible for the carcinogenesis. For many years it has been recognized that workers engaged in the manufacture of dyestuffs had a higher incidence of cancer of the bladder, "aniline cancer," than comparative groups of the general population. Yoshida (8) in 1932 first produced a liver cell carcinoma in rats following oral administration of an azo dye. Subsequently Japanese researches (3) have shown that a number of simple azo compounds have carcinogenic properties; *i.e.*, 4′-amino-2,3′-azotoluene (o-aminoazotoluene) which gives liver cell tumors when fed to rats, 2,3′-azotoluene which gives malignant urinary bladder tumors when administered orally to rats, and p-dimethylaminoazobenzene which produces hepatomas and cholangiomas in the liver of rats. Shear (7) obtained liver cell carcinomas in the M (leaden) and A strains of mice following *sub cutem* injections of approximately 70 mgm. of o-aminoazotoluene. It has also been shown (1), following the same technic, that this same compound induces lung tumors in mice of strains A and C, both susceptible strains.

These results are of extreme interest because of the use of certain azo compounds as food coloring matter, as healing agents in salves, and in specialized dyeing processes and the common usage of p-amino benzene sulfonamide (sulfanilamide) and related products in combating various types of infection.

Materials and Methods

The two inbred strains of mice used for a study of the effect of simple azo compounds on internal tumors were the C57 black strain and the dilute brown Dba strain, both from the Roscoe B. Jackson Memorial Laboratory. The C57 black strain has a relatively high incidence of internal tumors (4). Nonepithelial tumors occur in approximately 14 per cent of the animals and a high percentage of these are liver tumors, vascular in origin. The epithelial tumors arising in this strain are limited chiefly to the liver. The dilute brown strain, on the other hand, is a line with high incidence of mammary carcinoma with comparatively few internal tumors other than those involving the spleen and lymph nodes. Tumors of the connective tissue are extremely rare in both strains. Two sublines of the C57 black strain, VI and X, and subline IV of the Dba strain were used exclusively. Equal numbers of mice of both sexes were used in each experiment.

The four azo compounds used, o-aminoazotoluene, p-dimethylaminoazobenzene (butter-yellow), 4′-hydroxy-2,3′-azotoluene, and 2,3′-azotoluene, were obtained from the Eastman Kodak Co. and used without further purification (Fig. 1). The compounds were dissolved in a good grade of commercial olive oil and injected subcutaneously into the right axillary region of mice 2 months of age. A total of 5 mgm. was injected in 3 doses during the first 2 months. At 4 months of age a 5 mgm. pellet was inoculated by sterile trocar, making a total dosage for each animal of 10 mgm. Mice were kept as virgins.

Results

There appeared in a C57 black animal 105 days of age, 45 days following initial injection of o-aminoazotoluene, a subcutaneous growth which proved to be a fibrosarcoma. Of 30 mice which lived beyond the time of appearance of the first tumor, 13 (43.3 per cent) developed fast growing fibrosarcomas. The first fibrosarcoma in the butter-yellow series appeared in a C57 black mouse 112 days of age. In all, 6 of 29 mice in this series developed fibrosarcomas. At 175 days and 322 days respectively subcutaneous growths appeared in the 4′-hydroxy-2,3′-azotoluene and in the 2,3′-azotoluene series. Eleven of 30 (36.6 per cent) C57 black mice in the former and 5 of 20 (25 per cent) C57 black mice in the latter series developed subcutaneous growths. A total of 35 subcutaneous growths, all fibrosarcomas, appeared in 109 injected mice of the C57 black stock (Table II). Sarcomas arising in each of the four azo series produced 100 per cent takes of

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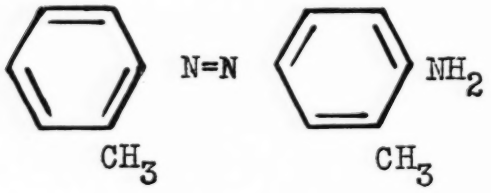
fast growing tumors when transplanted to the same strain.

The mean tumor incidence of the o-aminoazotoluene group was significantly higher than the mean tumor incidence of the butter-yellow series ($\bar{X}_D = 4 \times \sigma_D$)[1] and also significantly higher than the 2,3′-azotoluene series ($\bar{X}_D = 3 \times \sigma_D$).

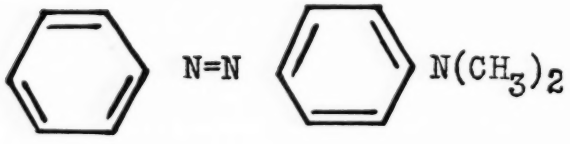
The mean latent period of fibrosarcoma induction in the o-aminoazotoluene series was significantly shorter than the mean latent period of any of the other three azo compound series. The differences of the mean latent periods between this group and the butter-yellow, 4′-hydroxy-2,3-′azotoluene, and 2,3′-azotoluene groups are 15.2 ± 3.9, 16.9 ± 3.8, and 21.9 ± 3.0 weeks respectively. No significant differences of the mean latent periods exist among the other azo groups.

Only one fibrosarcoma appeared among 106 mice of the Dba strain similarly treated and this appeared at 476 days in the butter-yellow series.

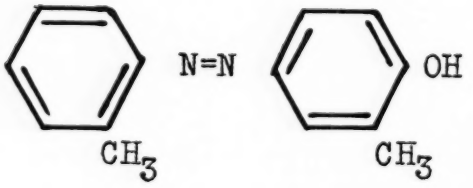
No tumors involving the urinary bladder or digestive tract were observed and the incidence of other tumors, chiefly lymphoblastoma in the C57 black strain and lymphoblastoma and breast tumors in the Dba strain were not significantly different from the spontaneous incidences given for these strains.



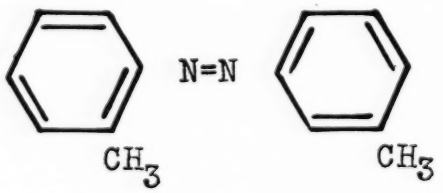
o-aminoazotoluene



p-dimethylaminoazobenzene



4′-hydroxy-2,3′-azotoluene



2,3′-azotoluene

FIG. 1.—Structural formulæ of the azo compounds used in these experiments.

LIVER LESIONS

Thirteen hepatomas were found in the experimental series, 9 of these in the Dba strain in which internal tumors exclusive of lymphoblastoma are comparatively rare and 4 in the C57 black strain which has a relatively high incidence of liver tumors (Table II). The majority of these were observed in the o-aminoazotoluene series.

There were no visible macroscopic changes in the livers of mice which died or were sacrificed within 2 months following initial injections. Histologically there was a distinct swelling of liver cells with a hyperchromatic appearance of nuclei. There also appeared in localized regions large dark staining hepatic cells, some with extremely large nuclei, others binucleate. Following these changes there appeared regions of local fatty degenerative infiltration in advanced condition macroscopically visible in the form of a definite nodule (Figs. 4 and 5). In later necropsies there were evident regions of definite necrosis with apparent regeneration around these areas of hepatic tissue in the form of hepatic adenomas.

[1] $\bar{X}_D$ = Difference of means; σ_D = Standard error of the difference.

DESCRIPTION OF FIGURES 2 TO 6

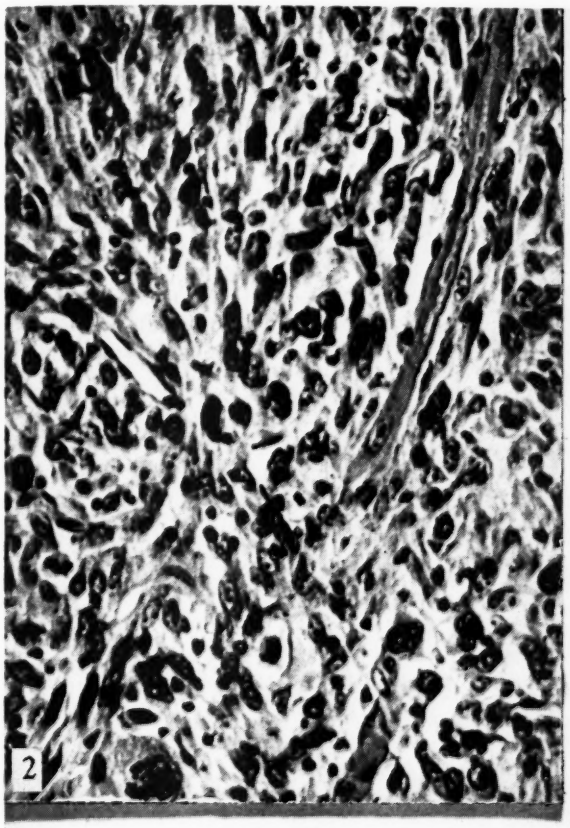
FIG. 2.—Fibrosarcoma in C57 black mouse following subcutaneous injection of o-aminoazotoluene. × 300.

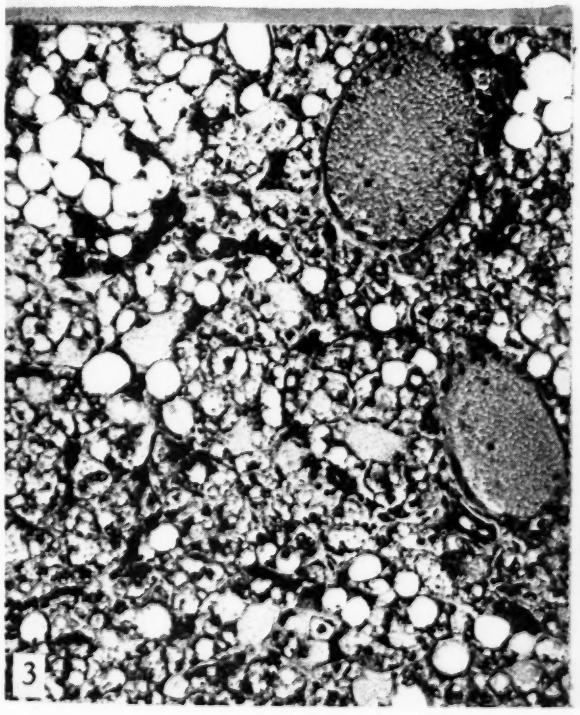
FIG. 3.—Fibrosarcoma in C57 black mouse following subcutaneous injection of butter-yellow. × 300.

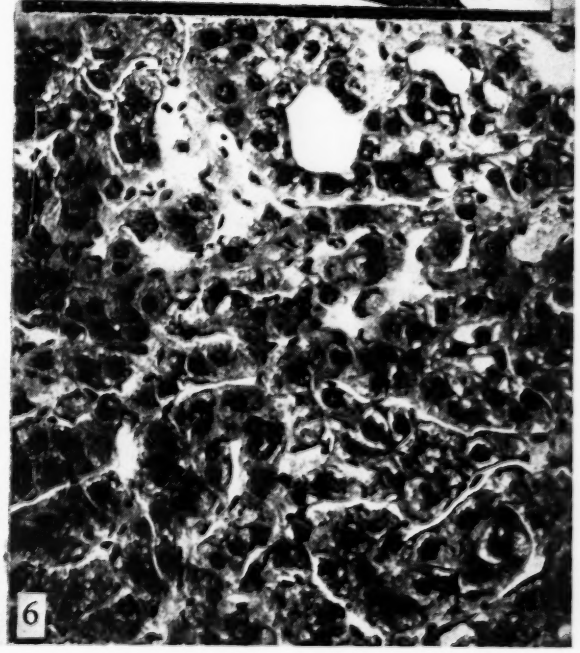
FIG. 4.—Nodule of liver in Dba mouse following subcutaneous injection of o-aminoazotoluene. × 20.

FIG. 5.—Same as Fig. 4. × 160.

FIG. 6.—Hepatoma in Dba mouse following subcutaneous injection of o-aminoazotoluene. × 200.







FIGS. 2-6

All liver cancers observed were trabecular hepatomas. There was found only slight adenomatosis of the bile ducts and this condition was by no means general. Two hepatomas were associated with reticulo-endotheliomas involving the spleen, mesenteric-intestinal lymph nodes, and liver.

Unlike the changes occurring in the liver of rats there was little or no proliferation of fibrous connective tissue nor was there extensive proliferation of bile duct epithelium toward cholangioma formation. Likewise tumor nodules were not multiple; not more than 3 nodules were found in any one liver.

Table I: Induction of Fibrosarcomas in Mice Following Subcutaneous Injections of Azo Compounds

Azo compound	Strain of mice	Number	No. of fibrosarcomas	Per cent fibrosarcomas	Mean age at appearance of tumor	Mean age at necropsy: Tumorous mice	Mean age at necropsy: Nontumorous mice
o-Aminoazotoluene	C57 black	30	13	43.3	264.6	281.7	551.6
	Dba	30	0	...	...	...	474.6
p-Dimethylaminoazotoluene	C57 black	29	6	20.7	357.9	385	544.4
	Dba	30	1	3.3	476	476	540.1
4′-Hydroxy-2,3′-azotoluene	C57 black	30	11	36.6	350.6	396.5	529.2
	Dba	24	0	...	...	...	513.5
2,3′-Azotoluene	C57 black	20	5	25	361.2	440.1	583.5
	Dba	22	0	...	...	...	588.6

Table II: Liver Lesions in Mice Following Subcutaneous Injections of Azo Compounds

Compound	Strain of mice	Age at necropsy	Lesion
o-Aminoazotoluene	Dba	434	Hepatoma
o-Aminoazotoluene	C57 black	465	Hepatoma, reticulo-endothelioma
o-Aminoazotoluene	Dba	591	Hepatoma
o-Aminoazotoluene	Dba	591	Fatty degenerative infiltration, hepatoma
o-Aminoazotoluene	Dba	591	Hepatoma
o-Aminoazotoluene	Dba	591	Hepatoma, adenomatosis of bile ducts
o-Aminoazotoluene	Dba	636	Hepatoma
o-Aminoazotoluene	Dba	654	Hepatoma with metastases, reticulo-endothelioma, adenomatosis of bile ducts
p-Dimethylaminoazobenzene	Dba	581	Fatty degenerative infiltration, hepatoma
4′-Hydroxy-2,3′-azotoluene	C57 black	604	Fatty degenerative infiltration, hepatoma
4′-Hydroxy-2,3′-azotoluene	Dba	629	Hepatoma
2,3′-Azotoluene	C57 black	585	Hepatoma, reticulo-endothelioma
2,3′-Azotoluene	C57 black	639	Fatty degenerative infiltration, hepatoma, lymphoblastoma

Discussion

Although the azo compounds have been used extensively both in feeding and *sub cutem* injection in rats by the Japanese (3) and to a lesser extent in mice (1, 2, 7), subcutaneous growths have not been commonly encountered. Schmidt (6) observed an infiltrating sarcoma on the neck of a mouse fed a Sudan dye. This appeared rather late in life and may have been spontaneous. A single sarcoma, partly fibrosarcomatous and partly reticulosarcomatous, was produced late in life following subcutaneous injections in the rat of butter-yellow (3). Failure to obtain subcutaneous tumors in mice may have been due to selection of resistant strains.

In the above experimental series of mice o-aminoazotoluene was more effective in inducing both fibrosarcomas and hepatomas than the other three azo compounds used. Substitution of OH for NH_2 in the azotoluene molecule (4′-hydroxy-2,3′-azotoluene) did not significantly change the incidence of induction of sarcomas but there did result a statistically significant lengthening of the latent period. If the amino or hydroxy group is removed from the molecule there results a statistically significant decrease in sarcomas induced as well as a significant lengthening of the latent period.

Perlmann and Staehler (5) were of the opinion that substances were carcinogenic if they had aminoazobenzene in their structure and that the carcinogenic effect would be lost if H of NH_2 were substituted for other groups. However, the N-methylated compound, butter-yellow, used in this study did induce sarcomas but the carcinogenic effect as measured by tumor incidence and latent period apparently was significantly decreased by such substitution.

SUMMARY

1. Thirty-five fibrosarcomas have been induced in 109 mice of the inbred C57 black strain of mice following injections *sub cutem* of 10 mgm. of four related simple azo compounds.

2. In these experiments with mice, o-aminoazotoluene (4′-amino-2,3′-azotoluene) was a more potent carcinogen in inducing both sarcomas and hepatomas than p-dimethylaminoazobenzene, 4′-hydroxy-2,3′-azotoluene, and 2,3′-azotoluene.

3. Only one sarcoma appeared in 106 mice of the Dba strain similarly treated, this in the p-dimethylaminoazobenzene series.

4. The chief liver reaction in the mice used in this experiment was the formation of trabecular hepatoma with only a slight adenomatosis of bile ducts and little or no proliferation of connective tissue.

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Cutaneous Neoplastic Responses Elicited by Ultraviolet Rays in Hairless Rats and in Their Haired Litter Mates*

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It is generally agreed that the melanin located within the epidermis and in subepidermal melanophoric cells, as well as the keratin contained in the hairs and in the horny layer covering the epithelium, provides an effective means of protection against the injurious and particularly carcinogenic action of natural or artificial ultraviolet rays. It is well established that fair-skinned and blond individuals are much more susceptible to the development of solar cancer of the skin than more heavily pigmented individuals, especially the colored races. Similar observations in this respect have been made by Rusch and Baumann (5) in regard to the relative sensitivity of albino and black mice exposed to ultraviolet rays. Watkins-Pitchford (6) stated that the susceptibility of man to cutaneous cancer as a result of exposure to sunlight is due to the lack of a hairy coat.

Interpretations of experimental actinic carcinogenesis in rats and mice have been used in recent years to minimize the significance of the role which solar rays play in the production of cancers in the human skin. Beard, Boggess, and von Haam (1) as well as Luce-Clausen (3) contended that the positive results, relatively easily obtainable in albino rats and mice, are not applicable to man, as not only the dose of ultraviolet rays administered for the production of the experimental cancers is disproportionately high in comparison to that practically encountered by man under environmental conditions, but also the skin of these animals, which are used to a dark habitat, is much more sensitive to ultraviolet rays than the human skin, thus leaving to man a wide margin of safety.

In view of the practical significance of this question, it was decided to make a comparative experimental study of the susceptibility to cancer of the skin of hereditarily hairless rats and of that of their haired litter mates when exposed to ultraviolet rays. The skin of the hairless rats resembles that of man with respect to keratin.

* Read at the 34th Annual Meeting, American Association for Cancer Research, Chicago, Illinois, April 16, 1941.

Experimental

The hairless rats used in this investigation were obtained by breeding male rats of a hereditarily hairless strain of the rhinoceros type against females of the albino Wistar strain. The dark grey haired females of the resulting litters were then bred with the original hairless males. While all of the offspring thus obtained developed initially either a white, dark grey, or hooded pelage, one fifth of the animals started to shed their hairs in the 4th or 5th week and became hairless usually during the 6th week, remaining in this condition throughout life. Animals of this heterozygous generation were employed.

The skin of the hypotrichotic rats which in the beginning is smooth and tightly fitting later becomes increasingly loosely attached to the body, forming folds, especially around the legs. Numerous protruding nodes of varying size appear in the skin. The eyelids become swollen and the ears thickened. In addition to the absence of hairs the epidermis assumes a greyish color with brownish spotting, and is covered by a dry, scaly, or cheesy keratinous material. Warty, hyperkeratotic excrescences surround the openings of the hair canals.

Apart from the abnormally marked keratinization, there is usually an increase in the cellularity of the epidermis which consists often of from four to six layers instead of the normal two or three. The normally straight contour of the epidermis is interrupted in the hypotrichotic rats by the frequent occurrence of deep sacculations representing distended hair ducts. These utriculi are filled with concentrically arranged cornified lamellae. Numerous smaller or larger epithelial cysts formed from dilated hair canals, hair follicles, and sebaceous glands and lined by one or two layers of flattened epithelial cells are found in the subepidermal connective tissue. The cysts contain circumferentially arranged keratin lamellae.

While the skin of these genetically malformed rats is not structurally identical with the human skin, it resembles the human epidermis in two important aspects: (a) there is an absence or great scarcity of

hairs; (b) the epidermis is covered by a thick keratinous layer. Inasmuch as previous experiments on actinic skin cancer have brought out the fact that the scantily haired parts of the skin with only a thin cornified layer and epidermis; *i.e.,* ears, nose, conjunctiva, forefeet, etc., are most susceptible, the use of hypotrichotic rats permits the study of the relative sensitivity of a heavily cornified and hairless skin to the carcinogenic action of ultraviolet rays.

Thirty-six hairless rats (33 females, 3 males) and 24 haired litter mates (all males), which were from 3 to 5 months old, were exposed to the radiation emitted from the mercury vapor burner of a Hanovia Super S Alpine Lamp yielding 1,500 milliwatts per square centimeter at a distance of 30 inches. At the start of the experiment the burner was placed 42 inches from the bottom of the wire cage in which the animals were kept during the treatment. The cage was of approximately the same size as the reflector of the lamp. The initial exposure of 2 minutes was gradually increased during 3 weeks to 60 minutes administered on 5 days weekly, while the distance of the burner from the bottom of the cage was reduced simultaneously to 25 inches. These conditions of exposure remained unchanged until the end of the experiment, which lasted for 10 months. At its conclusion the surviving animals were killed.

During the early part of the experiment 5 rats were kept in one cage. While no complications were encountered in connection with the haired rats, after the exposure to ultraviolet rays the hairless animals became violently aggressive, attacking mainly the tails of their cage mates. As a result, several fatalities were sustained among the hairless rats, which were thereafter kept in individual cages. The hairless animals proved to be very much less resistant to radiation in general than the haired rats. There were 25 deaths among the former and 5 deaths among the latter during the first 6 months of the experiment. All these animals died without showing signs of neoplastic responses of the skin of any kind. However, the skin of the hypotrichotic rats often displayed large rhagades and dry, thin, atrophic spots sometimes complicated by small ulcerations. The ears were thickened and the tips of the tails were frequently necrotic. During the latter part of the early period, the ears of the haired rats exhibited dry keratotic excrescences and general reddening and swelling. Similar changes, as well as a dry scaly eczema, were found on the nose, the eyelids, and the forefeet of some of these animals.

It was not until the 7th month that the first definitely neoplastic reactions appeared in the form of cornified horns and verrucous papillomas involving first the ears and later, in increasing number, other parts, such as the eyelids, nose, and forefeet of the haired animals, normally little protected by hairs. The corresponding responses in the hairless rats were always less pronounced in size and frequency. This discrepancy between the two groups of animals became increasingly marked toward the end of the experimental period when the haired rats were bearers of large cancers. The hairless rats still showed only a few small horns and numerous horny frills affecting the edges of the ears. The developments of the latter part of the experimental period, with data concerning deaths and neoplasia, are presented in Tables I and II.

Keratinized horns and nodes were especially frequent on the edges and inner surface of the ears, where they were often large as well as numerous. Fungoid and ulcerating cancers possessing a tissue with baconlike texture usually involved the orbit, and in two instances, the forefeet. This type of neoplasm was found

Table I: Mortality Table Showing Deaths During Months after the Beginning of the Experiment

	Months					Survivors
	1 to 6	7	8	9	10	
Hairless rats	25	2	1	2	5	1
Haired rats	4	2	1	4	1	12

Table II: Neoplastic Reactions Stated in Numbers of Lesions

	Reactions at seventh month			Reactions at tenth month		
	Keratoses, frills	Horns	Cancers	Keratoses, frills	Horns	Cancers
Hairless rats	11	2	..	6	..	1
Haired rats	19	6	1	13	6	13

only in haired rats. Two of these rats also exhibited metastatic nodes in the regional lymph glands at the base of the ears, and one rat had neoplastically enlarged abdominal and thoracic lymph nodes. The spleens of the haired rats were often large. The testes were not infrequently small, soft, atrophic and contained subcapsularly located white spots and streaks. During the course of the experiment, the skin of the hairless rats became increasingly thickened, wrinkled, dry, scaly, crusty, keratotic, and dirty brown and was usually covered by a thick, cheesy layer which seemed to loosen and swell after the skin was placed into formalin solution.

Tumors in haired rats.—Of the 20 haired rats examined histologically, all of which died during the 7th to the 10th month of the experiment, only the 2 animals which died during the 7th month were found free from malignant neoplasia. All others had one and most of them had numerous cancers. In 14 of these rats there were sarcomas in addition to multiple squamous cell carcinomas. While the majority of the carcinomas were of the cornified squamous cell type, some were very immature in structural development

and diffusely infiltrative. A few of the carcinomas exhibited morphologically a close resemblance to adamantinomas. The sarcomas were spindle cell, round cell, or polymorphous in type. Sarcomas of the polymorphous variety usually contained a considerable number of huge, indistinctly outlined giant cells with very large, irregularly shaped, hyperchromatic nuclei. In 2 rats two different types of sarcomas were observed, while in a third rat a spindle cell carcinoma of the skin coexisted with a spindle cell sarcoma of the subcutaneous tissue. Collision tumors consisting of cutaneous carcinomas and subcutaneous sarcomas involving the ears were found three times.

In addition to the definitely malignant reactions there were numerous cornified squamous cell papillomas and verrucous formations which originated from the generally thickened and hyperkeratotic epidermis of the haired rats. Small, localized, atypical epithelial proliferations or extensive atypical epithelial coating were frequently seen in these animals. Marked inflammatory reactions, when they ocurred, were present mainly underneath and near ulcerated lesions. There was, on the other hand, a marked vascularity and hyperemia present in many of the hyperplastic lesions, indicating that chronic inflammatory irritation and chronic cicatricial ischemia did not play any significant part in their genesis. The skin of the hairy parts of the body was normal.

Tumors in hairless rats.—At first, the epidermis of the hairless rats usually reacted with a thickening of the cornified coating and with an increase of the epithelial layers, the more superficial of which assumed a squamous cell character. Apart from these hyperplastic and hyperkeratotic lesions there occurred also atrophic, degenerative, and ulcerative changes. These were characterized by a thinning of the cellular layers, a shrinkage of the cells, a vacuolization of their cytoplasm, and ultimately a complete breakdown of the cellular structures. The connective tissue underneath these lesions, which were especially frequent at the tips of the epidermal folds, was often highly hyalinized. The connective tissue underlying the proliferative epidermal responses was, on the other hand, usually exceptionally vascular but free from any inflammatory reactions. A more or less diffuse filiform papillary proliferation of the epidermis appeared not infrequently in some parts of the skin, especially the ears, during the later stages of the experiment. The outer surface of the external ears was in general much less affected than the inner portion and the region of the tip. It was remarkable that the epithelial lining of the numerous epithelial cysts embedded in the subcutaneous tissue participated not infrequently in the proliferative and hyperkeratotic changes of the surface epithelium covering them. While there were sometimes short or plump or pointed pegs of epithelial cells extending into the subepithelial tissue from the hyperplastic epidermis, they assumed a malignant character in only one instance. This single cornified squamous cell carcinoma of a verrucous type was found upon microscopic examination of a growth at the base of the ear of one of the irradiated hairless rats which died during the last month of the experiment.

In addition to the discrepancy existing between the two groups of rats in regard to the type and number of preneoplastic and neoplastic responses obtained, significant differences occurred in the extent of the vascular lesions found in the internal organs of these animals, indicating variations in the effectiveness of the ultraviolet rays. During the histological study of the internal organs of the irradiated rats it was noted that the arteries of various organs showed more or less extensive degenerative, calcifying, and occasionally proliferative changes increasing in frequency, extent, and severity with the duration of treatment given. The pulmonary artery and its intrapulmonary branches exhibited subendothelial as well as medial calcifications, usually assuming a polypous form. Some of the large and medium sized branches of this vessel displayed a vacuolated and hyalinized media. Some of the pulmonary arterioles had swollen walls and subendothelial hyalinizations. Similar degenerations (vacuolization, hyalinization, swelling, and occasional calcification of media, clumping of atypical nuclei, presence of histiocytes) were found in the epicardial and myocardial coronary arteries and were accompanied not infrequently by scattered regressive myocardial changes (hyalinization, vacuolization, calcification, fibrosis, interstitial edema). Corresponding medial degenerations were observed in the main renal artery and its intrarenal branches. While calcifications were rarely found in the wall of the renal arteries, calcium casts occurred frequently in the tubular lumina. Medial vacuolization and calcification were seen occasionally in the pancreatic and suprarenal arteries as well as in the aorta, where in one case a focal foam cell thickening of the intima was found. The most frequent and extensive hyaline and calcifying changes occurred in the arteries of the testis and epididymis. The walls of these vessels were often markedly swollen and completely calcified. Atrophic, degenerative, and calcifying lesions of the spermatogenic tubules usually accompanied the vascular manifestations.

While these arterial changes were present in both the hairless rats and their haired litter mates, they were more marked and more common among the animals of the haired series. A comparison of the types of vascular lesions occurring in the two groups of rats in the early and later parts of the experimental period is given in Table III.

Discussion

The data presented indicate that the skin of hereditarily hairless rats is less susceptible to the carcinogenic action of ultraviolet rays than the skin of their haired litter mates, in regions in which these are not protected normally by a sufficiently dense pelage. The variations in the structure of the skin of the two types of rats tested accounts to a large extent for this difference. While the thin cornified and cellular layers constituting the epidermis of the haired rats offer relatively little resistance to the penetration of the ultraviolet rays and thereby to the carcinogenic action of this agent upon the epithelial and mesenchymatous cutaneous tissues, the normally hyperkeratotic and thick epidermis of the hairless rat possesses a much more effective protective mechanism in this respect. It is apparently for this reason that the appearance of carcinomas in the skin of irradiated hairless rats is either prevented or greatly delayed and that the development of sarcomas from the more deeply situated and therefore still better protected connective tissue is eliminated. The human skin displays a similar reaction. If the evidence obtained in these experiments is applicable to man, it seems to follow that the resistance of human skin to the carcinogenic action of solar rays depends not only upon the intensity of pigmentation, but to an appreciable degree also upon the relative thickness of the horny layer covering the exposed parts of the skin. Individuals possessing a thin cornified layer and epidermis in addition to a weak normal pigmentation or to a deficiently reactive pigmentation are presumably most susceptible to ultraviolet rays. These observations suggest that statements concerning the comparative sensitivity of rat skin and human skin to cancerigenic ultraviolet rays cannot be of general nature, but must be confined to definite constitutional types.

The skin of the irradiated hairless rats presents several additional interesting aspects in regard to the alleged importance of certain congenital carcinogenic factors. The subepidermal tissue of the hairless rats is replete with epithelial cysts originating from distended hair canals, hair follicles, and sebaceous glands. These malformations did not exhibit any demonstrable special tendency toward a neoplastic transformation of their epithelial lining while under the influence of ultraviolet rays and do not seem to possess such a quality under normal conditions. The published reports of hereditary hypotrichosis of rats do not make any mention of the occurrence of spontaneous neoplasia in the skin of these animals (2, 4).

A noteworthy similarity exists between the wart-like excrescences of the skin of old hairless rats and certain keratoses observed in man. The localized hyperkeratoses of the rats do not appear to possess any neoplastic potentialities in contrast to those displayed by some of the human variety (tar and arsenic keratoses).

A particularly interesting aspect of these studies is represented by the relations existing apparently between the causes of the neoplastic and arteriosclerotic manifestations and their connection with the normal average life span of the two types of rats used. The morphological type of the arterial lesions as well as the parallelism existing between their frequency and severity on the one hand, and the incidence and extent of the neoplasia in the two sets of rats on the other hand, suggest that both changes are the result of the same causative agent, the ultraviolet rays. This conclusion is supported by the fact that in the irradiated organism ultraviolet rays generate vitamin D which in turn, when present in excessive amounts, produces calcifying arterial lesions of the type observed.

Furthermore, the observations made in regard to the neoplastic and arteriosclerotic reactions of actinic genesis are of importance in relation to the alleged dependence of cancer and arteriosclerosis on physiological processes of senescence. Roberts, Quisenberry, and Thomas (4), working with the same strain of hypotrichotic rats that was used in these experiments, found that the average life span of this animal is 360 days, while that of its haired litter mate is 555 days. Hairless rats thus seem to pass through a more rapid aging cycle than the haired litter mates. Therefore,

Table III: Types of Degenerative Vascular Lesions in Irradiated Rats

	Calcifications				Degenerations			
	1st to 5th month		6th to 10th month		1st to 5th month		6th to 10th month	
Location of lesion	Hairless rats (15)	Haired rats (3)	Hairless rats (10)	Haired rats (21)	Hairless rats (15)	Haired rats (3)	Hairless rats (10)	Haired rats (21)
Lung	5	3	7	10	1	1	2	3
Heart	..	..	..	2	2	1	4	14
Aorta	..	..	..	..	..	..	..	3
Kidney	..	..	1	2	4	2	7	17
Pancreas	..	..	..	1	..	..	1	2
Suprarenal	..	..	..	1	..	..	..	1
Testes	..	..	..	21	..	..	..	21
Epididymis	..	..	..	2	..	..	..	..

if the senescence theory is correct, hairless rats should be more susceptible to the development of these "old age" diseases and should acquire them at an earlier age than the haired rats, especially when they are exposed to the additional stimulus of a specific causative agent. Actually the haired rats having a longer life span displayed a greater susceptibility to these processes. Additional evidence is thereby supplied in refutation of the senescence theories of these disorders.

Summary and Conclusions

When hereditarily hypotrichotic rats and their haired litter mates were exposed to ultraviolet rays from a mercury vapor lamp, the animals of the haired series developed multiple carcinomas and sarcomas of the skin, while the animals of the hairless series reacted with an accentuation of the normal hyperkeratotic conditions and with the appearance of a moderate number of cornified horns. Only one of the hairless rats developed carcinoma of the skin.

This difference in susceptibility to actinic carcinogenesis displayed by the two types of rats is attributed to the differences in the anatomical structure of their skins. The skin of the hairless rats, normally thicker and more keratotic than that of the haired, provides a higher degree of protection against the ultraviolet rays.

The frequent coexistence of neoplastic and arteriosclerotic and arteriocalcinotic lesions in the irradiated rats suggests that both manifestations are the results of an excessive exposure to the actinic energy. It is uncertain whether the same photochemically generated agent is responsible for the development of both cancer and arteriosclerosis.

As the two reactions are appreciably more frequent and more severe among the haired rats having an average life span of 555 days, than among the hairless rats possessing an average life span of 360 days, it does not seem probable that physiological aging processes play any significant role in the production of the cancerous and arteriosclerotic lesions elicited in the irradiated rats.

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Reticulum Cell Sarcoma of the Rat Transferred Through Twelve Successive Passages in Animals of Related Stock*†

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This communication deals with a reticulum cell sarcoma, arising in the mesenteric lymph nodes of Wistar albino rats, which was successfully transferred through twelve passages by subcutaneous implantation into animals of the same strain.

The literature contains few reports of spontaneous tumors of the lymphatic system of rats. Bullock and Curtis (1) found 78 lymphosarcomas in 489 tumor-bearing animals with a greater incidence in the males. McEuen (2) observed only one similar tumor in an experimental group of 65 females and 10 males. Later he (3) reported, in a colony of about 4,000 rats, 99 tumors, of which 75 were found in females and 24 in males. Ratcliffe (4) observed two such tumors in 74 Wistar stock rats and one in 393 rats of an experimental colony.

Our strain of rats, in which the tumor herein described occurred spontaneously, was developed from 4 females and 2 males obtained in 1937 from the Wistar Institute. The colony has been maintained on a basal ration of Purina Dog Chow supplemented with fresh greens daily and meat scraps several times weekly. In 1938, tumors of the mesentery, arising at the ileocecal junction, were observed in animals between the ages of 10 and 18 months with a high incidence between 12 and 14 months. Tumor was found in 21 of 83 autopsied rats over 10 months of age. Sixteen of these 21 tumors occurred in males and the remaining 5 in females. The spontaneous tumors grew rapidly, extended to the contiguous lymph nodes, spread through the mesentery and proved fatal in 2 to 3 weeks. A few of them showed shotty masses which were distributed throughout the entire omentum and invariably were associated with a bloody ascites. Metastases occurred in the spleen, liver, kidneys, mediastinum, and lungs. No changes in the peripheral blood could be demonstrated in these animals. The gross appearance of the tumor was firm and grayish white, except where hemorrhagic necrosis was present. The necrosis was usually extensive. The tumors varied in size from 1.0 to 6.0 cm. in length and 0.5 to 1.5 cm. in thickness. Microscopically, the tumor cells were pleomorphic, being composed of large cells with a moderate amount of cytoplasm and large, clear nuclei, with one or two prominent nucleoli. A similar but much larger cell containing two or three nuclei was found, and small lymphoid cells. Varying numbers of mitotic figures were present (Fig. 1). Irregularly distributed among the cells was a reticulum composed of branching fibrils of varying thickness. These fibrils were well demonstrated by Gömöri's silver technic (Fig. 2).

TABLE I: TRANSFERS OF TEN ORIGINAL TUMORS

Original tumors	No. of animals inoculated	Age of animals in days	No. of successful transplants
D 1	9	Adult	0
D 17	10 *	Adult	0
W 17	9	30	0
W 21	7	24–28	0
DF 5	8	22	5 †
W 23	4	30	0
DF 3	5	30	0
DF 20	3	31	1
DF 27	4	23	0
DF 25	4	48	0

* 300 R. U. x-ray to 5 animals, 2 days before inoculation.
† One of these was used for successive transplants.

Transplants were made by removing the least necrotic portions of the tumors under sterile conditions and teasing them in a sterile petri dish. The material was minced until it could be aspirated through an 18 gauge spinal needle into a 10 cc. syringe. Approximately 0.1 to 0.2 cc. of the macerated tissue was needed for injection. The tumor material was deposited in the nuchal region by inserting the needle at the right lumbar region and pushing it subcutaneously to the elected site. When less material was introduced, successful transplants were not obtained. Tumor tissue from 10 animals (3 ♀, 7 ♂), treated in this manner, was inoculated into 63 male animals of the same strain, varying in age from 22 to over 365 days. The results are summarized in Table I. Only 2 tumors grew after

* Read at the 33rd Annual Meeting, American Association for Cancer Research, Pittsburgh, Penn., March 20, 1941.

† This investigation was aided in part by a gift from Mrs. Herbert DuPuy.

inoculation. One of the two successful transplants failed to grow on second transfer. The other successful transplant was transferred through 12 passages. Further transfers were unsuccessful as the tumors were permitted to grow *in vivo* too long before removal for inoculation. The extensive degeneration and necrosis which had occurred prevented successful transplantation. The use of x-ray before inoculation, mucin mixed with the tumor tissue at the time of inoculation, or double simultaneous inoculations of the tumor material did not speed up the growth of the transplant. One hundred and sixteen animals were inoculated and 42.2 per cent of takes were obtained, as shown in Table II. Successful inoculations were obtained when the tumor material was removed from animals after 2 or 3 weeks' growth and transplanted into other rats of the same strain, aged from 17 to 104 days. Only male rats were available and the genetic relationship was not followed. The most rapid takes occurred when the inoculations were made into animals about 30 days old. Such takes grew extensively over the dorsum of the thorax, became attached to the thoracic wall, and rapidly developed necrosis and ulcerations of the skin and underlying tumor mass. Death in the inoculated rats occurred between 7 and 40 days following inoculation, with an average of 18 days. Definite mortality percentage cannot be calculated, as many animals were killed for transfer material.

The tumor was transferable with difficulty. The animal's natural resistance to the growth of the inoculated tissue was evidenced by rapid fibrosis and a regression of tumor growths. The fibrous tissue, infiltrating the tumor, divided the tumor mass into small islands where the cells underwent degeneration. The tumor growth after transfer resembled the original in the gross and microscopic appearance, except for the presence of foreign body giant cells about degenerating tumor cells. Transfers of the tumor not only invaded the adjacent muscle tissue but led to metastases in the lung, liver, spleen, mediastinum, kidney, and bone marrow. Metastases, other than those in the mediastinum, were composed of areas of infiltrating cells, similar to those of the original tumor. Emaciation and profound anemia occurred in the inoculated animals. In some cases the erythrocyte count fell below a million cells per cu. mm. The erythrocytes of these animals clumped when diluted with Hayem's diluting fluid. There was a concomitant leukocytosis with a moderate

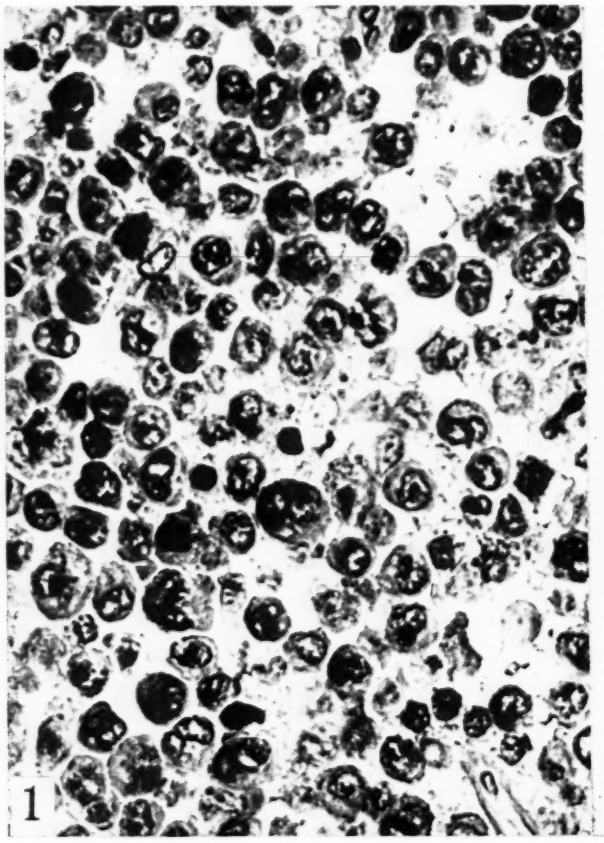

FIG. 1.—Section of inoculated tumor showing pleomorphic character of cells. Mag. × 800. Hematoxylin and eosin stain.
FIG. 2.—Section of same tumor showing character and distribution of reticulum. Mag. × 800. Gömöri's stain.

TABLE II: RESULTS OF SUCCESSIVE INOCULATIONS OF TUMOR

Generation of tumor	Age in days of tumor used for inoculation	No. of animals inoculated	Age in days of animals when inoculated	No. of takes	No. of deaths	Per cent of takes
1	7	8	23	5	0	62.5
2	7	6	24	5	2	83.3
3	13	7	30	6	3	85.7
	7	4	24	0	0	...
4	8	4	17	1	0	25.0
	8	14	23	6	1	42.8
5	10	10	27	4	4	25.0
6	21	4	20	3	1	75.0
	21	4	27	2	1	50.0
7	16	4 *	104	1	0	25.0
	16	4 †	27	2	1	50.0
	16	4	27	3	2	75.0
8	13	8 *	61	3	3	37.5
	24	2	20	0	0	...
	..	2	40	0	0	...
	14	2	23	0	0	...
9	..	2	40	1	1	50.0
	14	2	23	1	0	50.0
	14	4	42	0	0	...
10	24	2	38	0	..	...
	..	2	23	2	1	100.0
11	23	8	30	2	1	25.0
12	20	6	30	2	1	33.3
13	25	3	30	0	0	...
Total		116		49		42.2

* 400 R. U. x-ray.
† 200 R. U. x-ray.

or slight rise in the polymorphonuclears. Occasionally, a rise in the eosinophils to 8 or 10 per cent was found. The marked difference in the reaction of the hemopoietic system of the inoculated animals in comparison to those rats in which the tumor developed spontaneously is probably due in large part to the difference in age. Tumor could be transferred successfully only to rats aged between 2 and 4 weeks. Tumor never developed spontaneously in rats under one year of age. The inoculated tumors commonly grew more expansively than those developing spontaneously and the former frequently metastasized to the bone marrow where widespread fibrosis developed. It should be noted, however, that the rats with spontaneous tumor were few in number and many were killed before the growth became extensive and necrotic in order to remove tumor satisfactory for transfer.

SUMMARY

A reticulum cell sarcoma occurring spontaneously in the mesenteric lymph nodes was successfully transferred through 12 passages of related stock rats.

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Synthesis of Sterol by Tumor-Bearing Rats*

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Although many attempts have been made to show some correlation between malignant growth and sterol metabolism, the problem is still far from being solved. While certain phenanthrene compounds chemically related to sterols have been shown to be carcinogenic, Baumann and co-workers (1, 2) found that cholesterol when fed did not promote tumor growth, but, when applied locally in oil, might accelerate the development of cancer produced by such agents as ultraviolet light (7) or benzpyrene (2). The production of skin carcinoma in rats by ultraviolet light is preceded by a local concentration of cholesterol (6). Bergmann *et al.* (3), however, found that irradiated cholesterol did not act as a carcinogen.

If the initiation or growth of malignant tissue is accompanied by abnormalities of cholesterol metabolism, a well controlled study of the sterol balance might indicate a difference between normal and tumor-bearing animals. Breusch (4, 5) made a study of the sterol balance in normal and tumor-bearing mice but was unable to show any significant difference.

In preliminary tests it was found that it was impossible to interpret the results between normal and tumor-bearing rats unless the food intake of the normal controls was restricted to correspond with that of the tumor-bearing animals. In the present paper a carefully controlled study of the sterol balance has been made as a comparison between normal rats and those bearing implanted Walker adenocarcinoma 256.

Experimental

Young male Slonaker rats about 4 weeks of age were placed upon a low-sterol basal diet consisting of purified casein 20, cornstarch 55, dried yeast 8, Osborn and Mendel salt mixture 4, and Crisco 13, with the addition of Navitol [1] equivalent to 42.5 mgm. per 100 gm. of diet. The casein was purified by alcohol and ether extraction. Analysis of the mixed diet showed 0.13 per cent total sterol, which was furnished mainly by the Crisco (a hydrogenated plant oil) and yeast. Plant sterols are not readily absorbed from the alimentary tract (8).

After a period of 6 days on the low-sterol basal diet the animals were divided into groups of litter mates for experimentation, and fasted for 24 hours. During the period of fasting one of each pair of litter mates was implanted with Walker adenocarcinoma 256. The rats were weighed at the end of the 24-hour fast and the sterol in their bodies estimated from the data for Fig. 1.

The results of analyses made to establish a basis for estimating the amount of sterol in the bodies of young rats at the beginning of the experimental period are shown in Fig. 1. To obtain the data, young rats from the same colony were treated in the same manner as the experimental animals, including the 6-day feeding of the basal diet followed by 24 hours of fasting. The rats were then sacrificed and analyzed for total sterol. The total sterol content of young rats that have been treated in the same manner is uniform and shows a correlation with the body weight of the rat.

The animals were maintained in individual metabolism cages with screen bottoms and all excreta collected to be analyzed. The low-sterol basal diet was supplied in containers having a funnel-shaped opening to prevent scattering. The tumor-bearing rats were given food ad libitum. The food of each normal rat was restricted to correspond to that consumed by the tumor-bearing litter mate. The experimental period was about 2 weeks, and was determined by the rate of tumor growth and maintenance of appetite by the tumor-bearing rats. When the appetite began to fail the experiment was terminated by fasting the animals for 24 hours before killing and analyzing them. The excreta and carcass of each rat were analyzed separately.

Total sterol was determined by a method based upon that outlined by Schoenheimer and Breusch (9).

Results

As the food intake of each normal rat was restricted to correspond to that of the tumor-bearing litter mate, the sterol intake and gain in weight for the rats in each group are very close, as shown in Table I.

* This paper is based upon a thesis submitted by E. Elizabeth Troescher to the Faculty of the University of Washington, 1940, in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

[1] 55,000 A, and 10,000 D, U. S. P. XI units per gm.; furnished through the courtesy of E. R. Squibb & Sons, Seattle, Washington.

The results obtained from the sterol balance studies are given in Fig. 2. The increase in sterol in the body of a rat is calculated as the difference between the determined total sterol at the end of the experiment and the sterol estimated, from Fig. 1, to have been in the rat at the beginning of the experimental period. The increase in excreted sterol is calculated as the difference between the determined sterol in the excreta and the sterol ingested with the diet. The total increase in sterol, or the sterol synthesized during the time of the experimental period, may be obtained as the sum of the increase in the body of the rat and the increase in excreted sterol.

TABLE I: STEROL FED AND WEIGHT GAINED DURING BALANCE EXPERIMENTS

Unit	Rat *	Sterol fed, mgm.	Weight gained, gm.	Duration of experiment, days
1	10	131	12.6	15
	11	129	8.2	
	11A	128	9.0	
2	20	108	11.6	11
	21	93	4.6	
3	20A	127	11.4	14
	21A	125	11.1	
4	30	152	21.9	14
	31	152	22.7	
5	30A	128	8.5	14
	31A	128	7.5	
6	30B	141	13.0	14
	31B	140	17.9	
Average, tumor-bearers		128	11.6	
Average, controls		131	13.2	

* The first digit of each rat number represents the litter to which the animal belongs; if the second digit is a 1, the rat bore a tumor, while if the second digit is 0, the rat was a normal control.

The total sterol synthesized by a tumor-bearing rat was always significantly greater than the sterol synthesis shown by the normal litter mate control. The sterol synthesized varied from group to group as there was a difference in the rate of development of the implanted tumor tissue from one animal to another and consequently a difference in appetite, with resulting variations in the growth rate and duration of the experiment. The difference between the sterol synthesized by tumor-bearing rats and normal rats could be accounted for by the difference in the increase of body sterol, or of stored sterol. The sterol excreted was practically the same for the tumor-bearing and normal rats.

SUMMARY

Young rats bearing implanted Walker adenocarcinoma 256 and fed an adequate synthetic low-sterol diet consistently synthesized more sterol than normal litter mates, when the food intake of the latter was limited to conform to that of tumor-bearing litter mates.

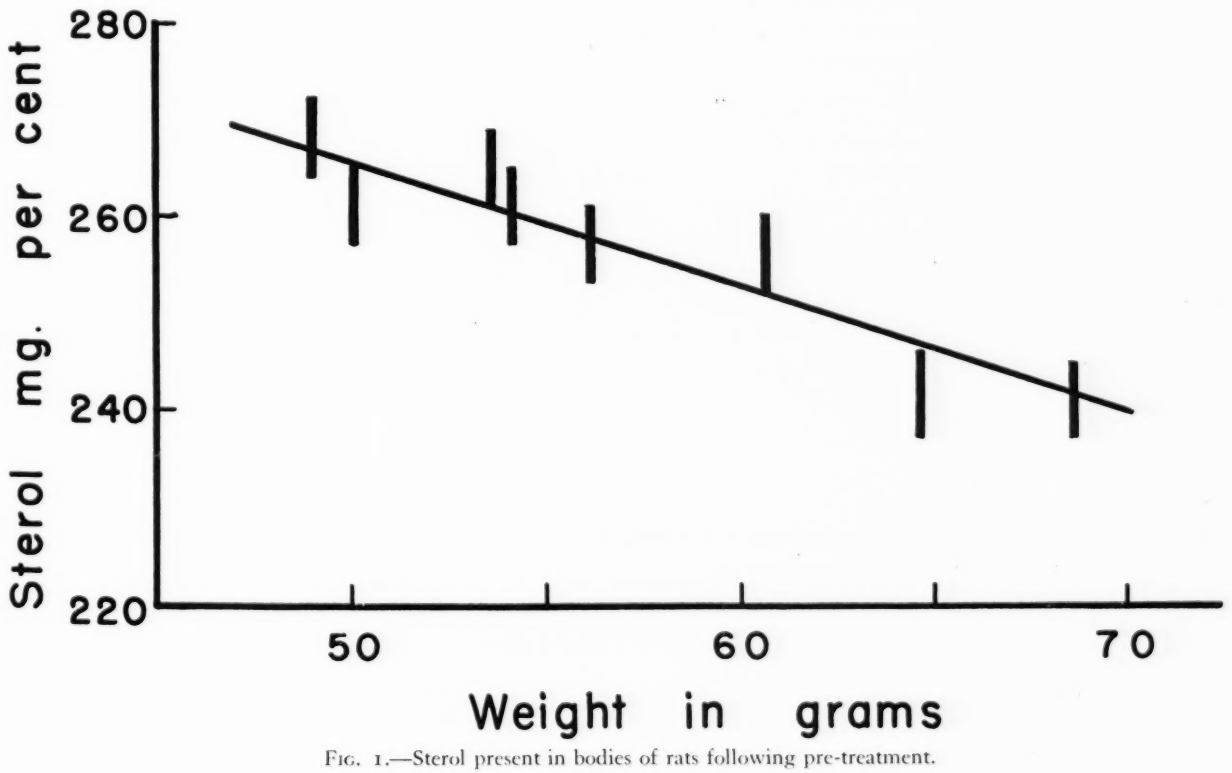

FIG. 1.—Sterol present in bodies of rats following pre-treatment. The range of experimental error is indicated by the vertical lines.

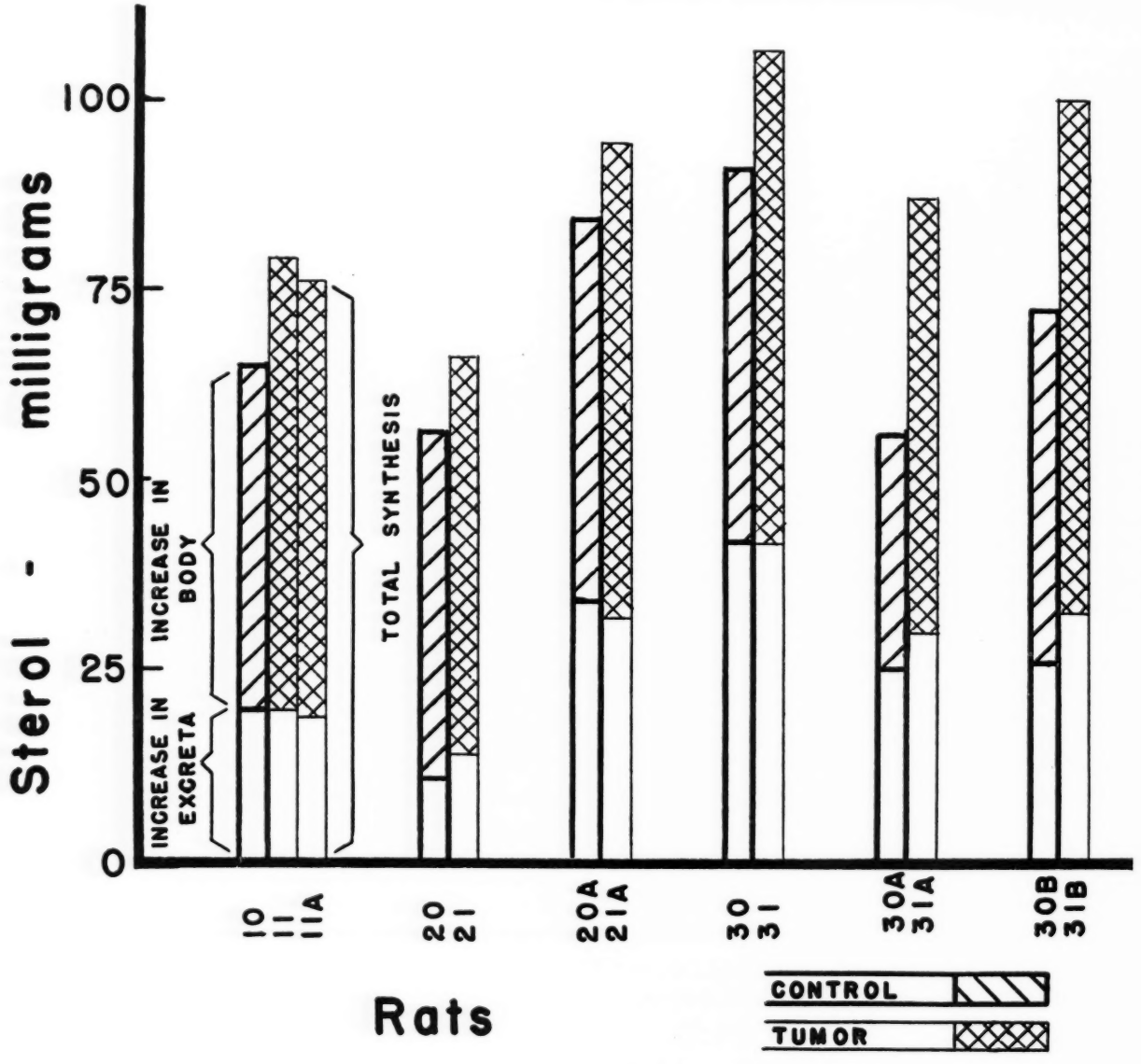

FIG. 2.—Synthesis of sterol by tumor-bearing and control rats.

Tumor-bearing rats excreted the same amount of sterol as normal control rats.

The significantly greater sterol synthesis shown by tumor-bearing rats was entirely accounted for by their greater increases in body sterol.

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Cancer Mortality in New York City: 1901 to 1938*

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Cancer, a Leading Cause of Death

Cancer now ranks second among the leading causes of death in New York City. The mortality ascribed to cancer is exceeded only by that charged to diseases of the heart, kidneys, and the circulatory system. Cancer has attained this position not solely because the recorded cancer death rate has increased, but also as a result of the reduction in the mortality from diseases which formerly had higher death rates than cancer. Prior to 1920, the standardized death rates from tuberculosis and pneumonia were consistently higher than that recorded for cancer today. In the past 30 years, the tuberculosis death rate has fallen almost 80 per cent and that from pneumonia more than 70 per cent. Since 1901, the standardized death rate from recorded cancer has increased 73 per cent.

Important as cancer now appears as a cause of death, it is probable that many deaths actually caused by malignant growths are not charged to this cause, either because the condition is not recognized or because the known facts are intentionally concealed. In New York City, steps have recently been taken to provide a method of registering deaths that will preserve the confidential nature of the reported cause of death and thus remove reasons for concealment. Experience elsewhere indicates that this procedure will result in the more frequent reporting of cancer as a cause of death when such a diagnosis has been made. However, this discussion of cancer mortality is restricted to the consideration of deaths reported in New York City as having been caused by the various forms of malignant growths included under the general term "cancer" in the International List of Causes of Death. This classification was adopted for use in New York City in 1901, and our study opens in that year.

Increase in the Recorded Cancer Mortality: 1901-1938

In New York City, in 1901, cancer was charged with 2,463 deaths and in 1938, the number of deaths ascribed to this cause was 11,160. In the first of these years, the estimated population of the city was 3,554,000 and in the latter, it was estimated to be 7,492,000. Even if the mortality rate had remained constant over this period, the *number* of cancer deaths in the city would have increased as a result of the general growth of the population.

Table I: City of New York. Cancer, All Forms, All Sites. Averages of Age Specific Death Rates, by Sex, 1901-1905 and 1934-1938

Age groups	Averages of age specific death rates			
	Males		Females	
	1901-05	1934-38	1901-05	1934-38
0-24	3.5	4.4	3.0	3.8
25-34	12.7	12.8	22.2	23.5
35-44	46.9	55.8	112.0	97.8
45-54	141.3	232.7	259.3	277.9
55-64	342.6	639.4	449.5	577.1
65-74	541.2	1216.9	634.0	980.0
75 and over	647.7	2051.4	704.7	1669.7

On the basis of the estimated populations and of the numbers of deaths ascribed to cancer in the city in 1901 and 1938, the *crude* death rates from this cause were, respectively, 69.4 and 149.0 per hundred thousand of population. The recorded increase in cancer mortality is, therefore, due to factors other than the increase in the population.

Age specific death rates by sex.—The first step in the search for the explanation of observed increase in cancer mortality was the analysis of the data by sex and age. The results of this analysis are presented in Table I. Similar data for a recent 3-year period are shown in Fig. 1.

This table and chart show clearly the wide variations in the cancer death rates at various ages, for each sex, and, to a lesser extent, the differences between the recorded mortality rates at each age in the two sexes. From these facts, it is obvious that the age and sex

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The data on which this paper is based were compiled, and the standardized death rates were calculated, by Irving Galloway, Junior Statistician of the Bureau of Records, under the direction of Mr. Louis Weiner, Chief of the Statistical Division.

composition of the population have an important bearing on the *crude* cancer death rate of a community. It is also obvious that before any reliable estimate of the trend of the true cancer mortality may be reached, the matter of the age and sex composition of the population must be examined, and, if possible, the effects of any differences or changes must be eliminated.

Effects of population changes.—Accordingly, the next step in the study of the cancer mortality was the examination of the age and sex composition of the population as recorded in the Federal censuses nearest the first and last years of our study.

The proportion of the population in the various age groups in New York City changed from 1900 to 1930. Under 25 years of age, cancer is not an important cause of death; consequently the decrease in population in these groups, and particularly the large decrease at ages under 10 years, does not greatly influence the cancer death rate. More important in their effect upon cancer mortality are the appreciable increases over 35 years of age and more particularly the large increases in the age groups over 45 years. In each of the three decennial age groups from 45 to 74 years, among both males and females, the population in 1930 showed an increase of more than 30 per cent over the figure for 1900.

From consideration of the age specific death rates and these observations on the changes in the age composition of the population in New York City, it is obvious that a substantial portion of the increase in the *crude* cancer death rate from 1901 to 1938 can be charged to this ageing of the population. In a paper not yet published (3) it is shown that 38 per cent of the increase in New York City in the *crude* male cancer death rate is directly due to the ageing of the male population; similarly, for females, 36 per cent of the increase in the *crude* cancer death rate may be ascribed to changes in the age distribution. To overcome the effect of changing age composition, and also to place the rates for the two sexes on a comparable basis, the observed age specific death rates for each sex have been adjusted to a standard population. As the standard, the population of England and Wales in 1901 was used. The New York City death rates for cancer of all forms and all sites, per hundred thousand persons of both sexes, and all ages, standardized on this basis, were 84.0 and 145.6, in 1901 and 1938, respectively.

The apparent increase in cancer mortality over this period, as shown by the standardized rates, is 73 per cent. This is considerably less than the increase indicated by the crude rates, because the effects of the advancing average age of the population have been eliminated in the standardization process. In the remainder of this presentation, discussion will be limited to standardized and age specific death rates. This means that any observed changes must be ascribed either to real changes in cancer mortality, to changes in diagnostic technics and the frequency of their use, or to changes in the methods of reporting and classifying causes of death.

Cancer Mortality by Sex

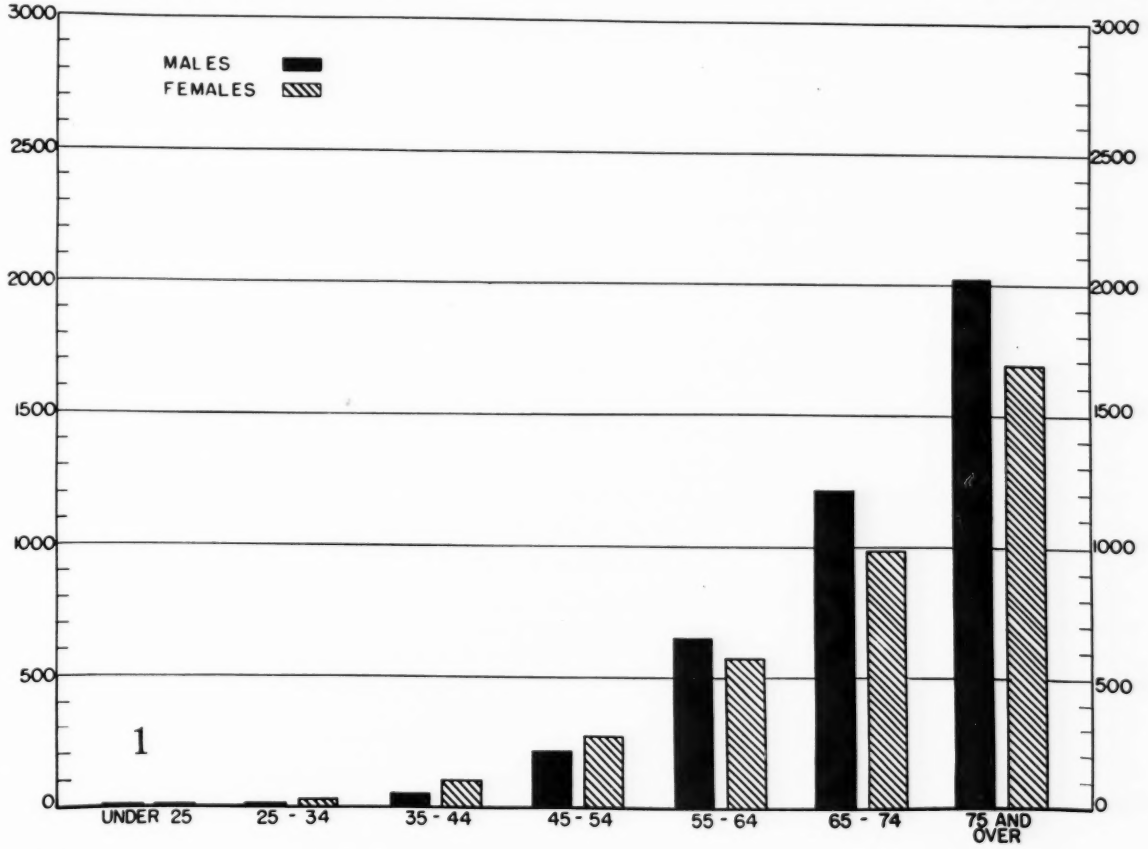
From 1901 to 1938, the standardized cancer death rate for males increased from 68.2 to 148.8 per hundred thousand, almost 120 per cent. In the same period of time, the standardized mortality rate from recorded cancer among females in the city rose from 100.4 to 134.4, an increase of only 34 per cent. The curves, showing how the male cancer death rate in New York City overtook and exceeded that among females, are shown in Fig. 2.

Ratio of mortality among males and females.—That cancer mortality is higher among males than among females, was recognized only recently. In 1901, 935 males and 1,528 females were reported to have died of cancer in New York City. In 1938, the reported cancer deaths among males numbered 5,602 while the number reported among females was 5,558. This was the first year in which the number of cancer deaths recorded among males was in excess of the number of female deaths charged to this cause in New York City. Although, in preceding years, the deaths charged to cancer were invariably more numerous among females than among males, in six of the years since 1929, and in each of the last 4 years, the male death rate has been the higher when the differences in the age distribution of the males and females in our population have been eliminated by standardization. On the basis of the standardized death rates, it appears that if the age distribution of the males and females in the City of New York were the same, in 1901, 68 male deaths from cancer were recorded for every 100 cancer deaths among females, while in 1938, 110 male cancer deaths were registered for each 100 female deaths. Furthermore, the curves in Fig. 2 appear to be following their general trends.

DESCRIPTION OF FIGURES 1 AND 2

Fig. 1. City of New York: cancer—all forms—all sites. Averages of annual death rates per 100,000, by sex and age, 1935, 1936, and 1937.

Fig. 2. City of New York: cancer—all forms—all sites. Standardized death rates per 100,000, all ages, by sex.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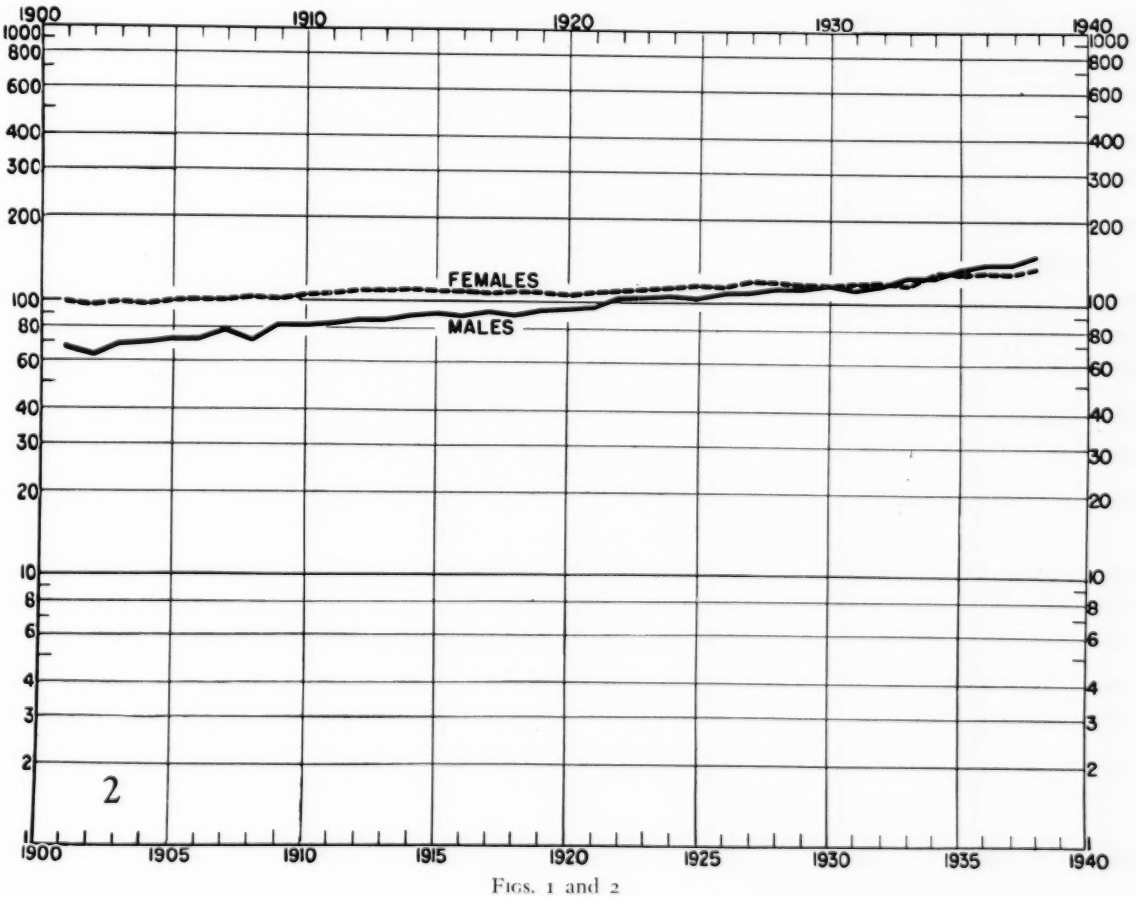
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FIGS. 1 and 2

Better diagnosis a factor in recorded increase.—These observations, however, prompt several suggestions. First, we are well aware that improved diagnostic technics, together with increased hospitalization, operations, and autopsies have resulted in more frequent reporting of cancer as a cause of death. Secondly, there may be a real increase in the incidence of cancer for certain sites. It may be that these two circumstances combine to raise the recorded cancer mortality rate or it may be that there has been no real increase in the incidence of cancer and that the observed increases in the recorded rates, after standardization, may properly be ascribed to the more frequent recognition of cancer from year to year. To offset these forces, there are two which would tend to decrease the cancer mortality rate: first, the effects of improved therapeutic methods resulting in cure; and secondly, a possible real decrease in the incidence of cancer of certain sites, such as that which may be responsible for the decline in recorded mortality from cancer of the uterine cervix. This decline can probably be partially explained on the basis of fewer births, and better obstetrical and *post partum* care.

Of course, in discussing these rates we must bear in mind that we are dealing only with deaths reported as having been caused by cancer. We can discuss the behavior of mortality rates based on these reports but we cannot interpret with any degree of certainty the extent to which the several forces, mentioned above, have influenced these rates. We have already described how the process of standardization eliminates the effect of age differences and age changes in the population upon the mortality rates of recorded cancer; we are now considering only the increase remaining after adequate allowance has been made for changes in the age and sex composition of the population. The differential increases in the mortality from cancers of various sites will shed further light on these points.

Mortality by Sex and Anatomic Groups

The standardized death rates from cancer, all forms, in New York City, by sex and site, for the years 1901 to 1938, are shown in Figs. 3 and 4. The values at the ends of each curve are presented in Table II, while the averages of the annual rates together with the trend of the annual rates for each site are shown in Table III.

Anatomic groups showing decreased mortality.—Over the entire period of this study, out of a total of twelve site rates (five for males and seven for females), only three decreases that were statistically significant [1] were recorded: among males, the skin; among females, the buccal cavity and the stomach and liver.[2] Among females, declining mortality rates were also recorded for the skin and the genital organs, but the rate of decline was not significant.

Although significant increases were recorded in the male death rates from cancers of the buccal cavity and cancers of the stomach and liver when the entire period 1901 to 1938 is considered, this was not the case for buccal cancers for the period 1905 to 1933, nor for cancers of the stomach and liver from 1918 to 1938. This leaves only two of the International List titles that have shown and are continuing to show increasing trends for both sexes over the period of this experience; namely, (a) the intestine and rectum and (b) the miscellaneous group,—other and unspecified organs. To these groups, essentially internal, must be added cancer of the breast among females.

Table II: City of New York. Cancer, All Forms, All Sites. Standardized Death Rates per 100,000, 1901 and 1938

Site	Males		Females	
	1901	1938	1901	1938
All sites	68.2	148.8	100.4	134.4
Accessible sites *	9.2	10.0	41.1	52.9
Inaccessible sites †	59.0	138.8	59.3	81.4
Buccal cavity	6.8	7.4	1.2	0.9
Stomach, liver	35.0	43.1	35.4	28.8
Peritoneum, intestines and rectum	9.0	32.1	12.6	29.8
Female genital organs	..	..	24.4	25.9
Breast	0.0	0.3	13.8	24.9
Skin	2.3	2.3	1.8	1.3
Other organs, or organs not specified	15.1	63.7	11.3	22.8

* Includes skin, buccal cavity, breast, female genitals.
† Includes stomach, liver, peritoneum, intestines, rectum, lung, pancreas, prostate, kidneys, etc.

Mortality from cancer of breast in females.—The encouraging picture that the increase in recorded cancer mortality may have been restricted to internal organs, and may have been due to better diagnosis, is marred by an almost uninterrupted rise in the recorded mortality from cancer of the female breast.

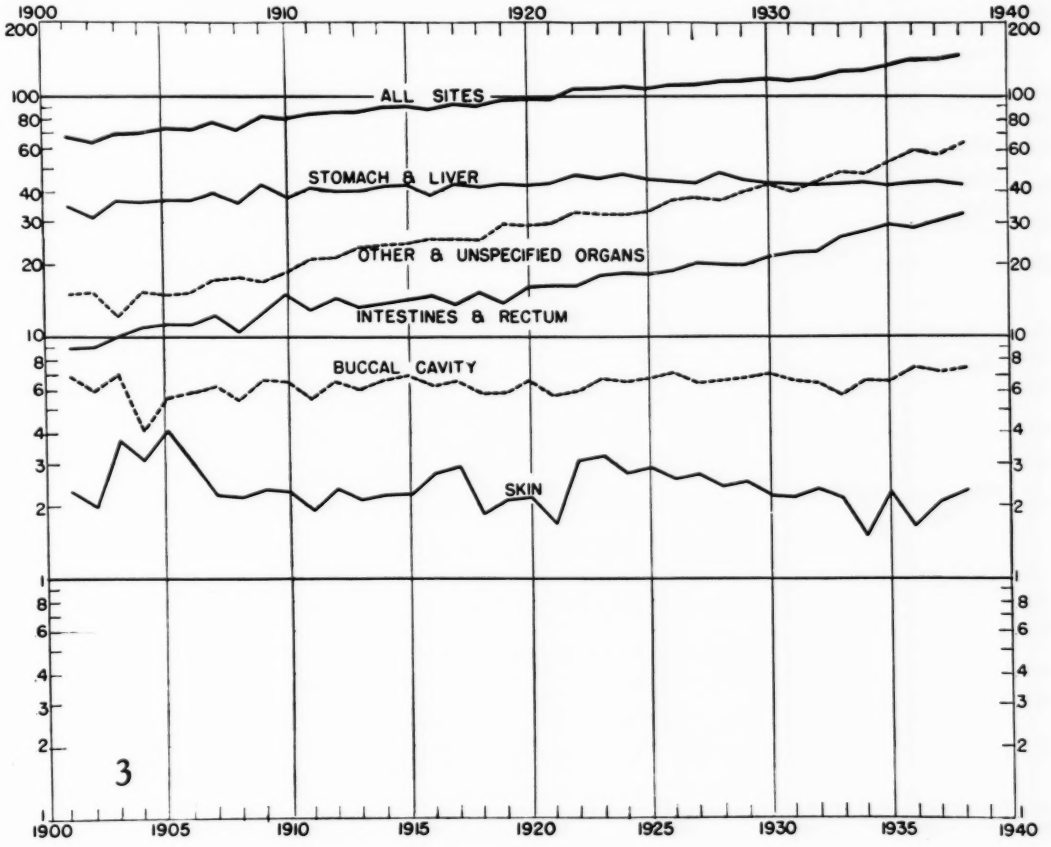
[1] See footnote to Table II.

[2] The stomach and liver are grouped in this discussion because they were so grouped in the International List of Causes of Death, prior to 1930. Mortality data for cancers of the stomach and liver, considered separately, are available for New York City only since 1930.

DESCRIPTION OF FIGURES 3 AND 4

Fig. 3. City of New York: cancer—all forms—all sites. Standardized death rates per 100,000, males, all ages.

Fig. 4. City of New York: cancer—all forms—all sites. Standardized death rates per 100,000, females, all ages.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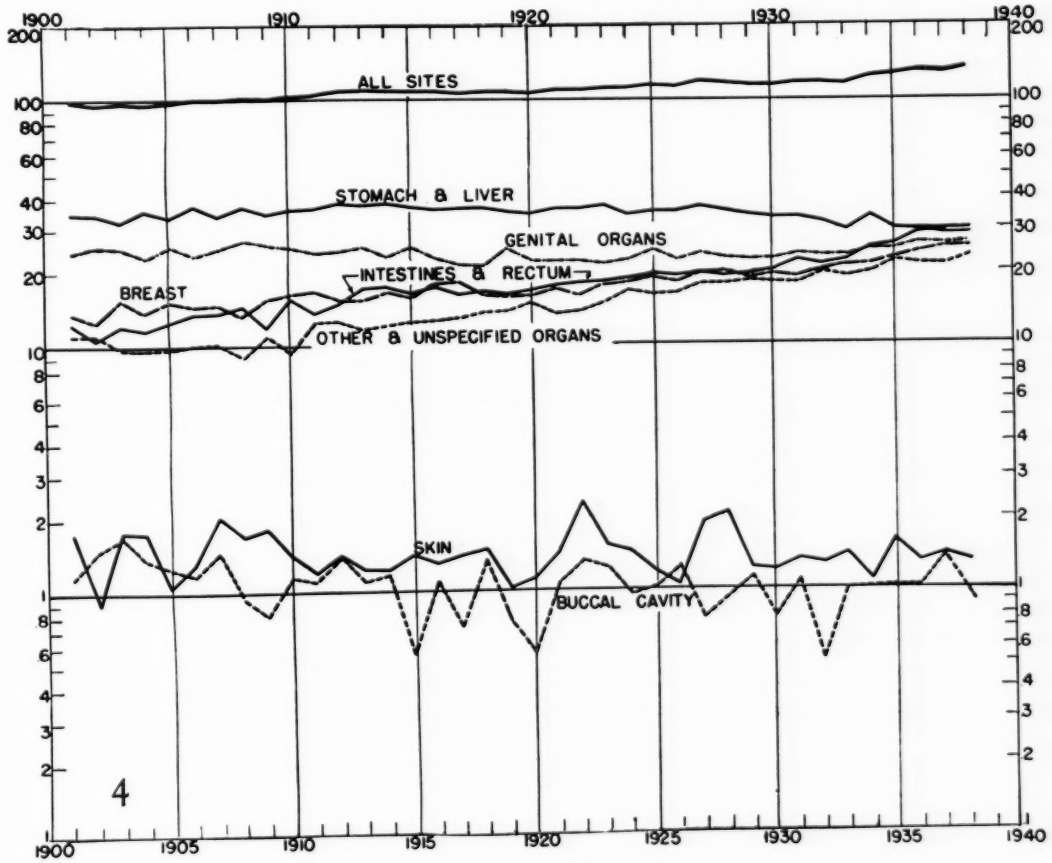
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FIGS. 3 and 4

The standardized death rate from mammary cancer among females was 13.8 in 1901, 16.6 in 1910, 16.4 in 1920, 19.6 in 1930, and 25.9 in 1938. By contrast, the rates for cancers of the stomach and liver among females for the same years were: 35.4, 36.4, 35.0, 33.6, and 29.1. From its peak in 1912, when the rate was 38.7, the *recorded* mortality from cancer of the stomach and liver among females declined 9.6 points, and in the same period, the recorded mortality for breast cancer increased approximately the same amount, 9.3 points. Is it possible that there is a relationship between the apparent decline in the death rate ascribed to cancer of the stomach and liver among females and the apparent rise in that from cancer of the breast? We think that it is possible and believe, with Bigelow and Lombard (1), that some of the recorded increase in the mortality from breast cancer, and the corresponding decreases in the death rate from cancers of the stomach and liver, have come about as a result of more accurate reporting on death certificates of the primary site of growth during recent years.

TABLE III: CITY OF NEW YORK. CANCER, ALL FORMS. AVERAGE STANDARDIZED DEATH RATES PER 100,000 AND TREND OF MORTALITY BY SEX AND SITE, 1901 TO 1938

Site	Averages of annual standardized death rates		Trend of mortality Average annual change (Per cent of average of rates)	
	Males	Females	Males	Females
All sites	99.3	113.1	+2.0	+0.8
Accessible sites *	9.1	44.7	+0.2 †	+0.5
Inaccessible sites *	90.2	68.4	+2.2	+1.0
Buccal cavity	6.4	1.1	+0.4	−0.9
Stomach, liver	41.8	35.1	+0.6	−0.4
Intestines and rectum	17.4	18.3	+3.1	+2.3
Female genital organs	..	24.3	..	−0.1 †
Breast	0.25	17.9	+2.6	+1.4
Skin	2.4	1.4	−0.7	−0.2 †
Other and unspecified	31.0	15.0	+3.9	+2.4

* See Footnote Table II. Excluding the mortality from breast cancer, the average of the standardized death rates from accessible cancers among females was 26.8 per 100,000, and the trend of mortality was −0.1, a nonsignificant decrease.

† Indicates nonsignificant trend.

NOTE: To determine whether the slope of the trend line departs significantly from the horizontal, "Student's" t is found from the expression

$$t = \frac{b}{\text{standard error of } b}$$

where b is the slope of the trend line, in the expression $y = a + bx$ and the standard error of $b = s_b = \sqrt{\frac{\Sigma y^2 - na^2 - b^2\Sigma x^2}{(n-2)\Sigma x^2}}$ and $\Sigma x = 0$.

From Table IV: (Fisher's *Statistical Methods for Research Workers*) for the value of t found by the above method, and $N = n - 2$, find the value of "P." When "P" $< .05$, it is assumed that the trend line departs significantly from the horizontal.

The apparent rise in the death rate from breast cancer among females in New York City, instead of reflecting an actual increase in incidence, may be due to understatement of the mortality from this cause in the early years of this experience, and the tendency toward more accurate reporting of the primary site in recent years. Decline in the death rate for cancer of the stomach and liver is compensated, in almost every age group, by a corresponding increase in the death rate from breast cancer.

Mortality from cancer of female genital organs.—The age distribution of the deaths from cancers of the female genital organs indicates that at ages below 55 the mortality from cancers of these sites, principally the uterine cervix, has declined steadily throughout the entire period of this experience. Evidence of increase was apparent only at the highest ages. The decline, particularly marked in the age group 35 to 44, has undoubtedly resulted from the declining birth rate, coupled with better obstetrical and *post partum* care on the one hand, and from appropriate surgery and radiology on the other.

Mortality from cancer of the digestive tract.—For cancer of the stomach and liver among males, there has been an increase only in the highest age periods, and even in these groups, there has been little indication of increase since the early 1920's. Among females, only in the age period 75 and over was the 1938 death rate from cancers of the stomach and liver higher than that of 1901. By comparison of the standardized rates, it appears that the mortality in males from cancers of the stomach and liver is approximately one-third higher than that for females. Below age 35, there appears to be little difference in the death rates from cancers of the stomach and liver in the two sexes, but from that age onward, there is an excess of mortality among males which amounts to 47.5 per cent in the age group 35 to 44, more than 50 per cent in the age group 45 to 54, and reaches its maximum of 83 per cent in the age group 55 to 64, after which it declines to less than 50 per cent. In this connection, it is interesting to note that although rates for cancer of the intestines and rectum have increased for both males and females at all ages over 35, the rates and trends are practically identical in the two sexes.

Mortality from cancers of miscellaneous sites—"other and unspecified."—Among males, cancers of the miscellaneous sites, not classified in other titles, include for the most part internal organs where malignant growths frequently advance beyond the possibility of cure before they are diagnosed. The group includes, among other sites, the following: respiratory system (larynx, lung, etc.), genitourinary system (kidneys, ureters, prostate, testes, etc.), as well as the brain, bones and joints, eyes, limbs, etc. The outstanding feature of the recorded mortality among males from cancers of these sites taken as a group, is that it has shown a constant and rapid increase at all ages.

The male rates of mortality from cancers included under this title are not comparable with the female rates, because the sites included differ in the two

sexes. For example, although cancers of the male genitals are included here, the cancers of the female genital organs are classified in a separate title. The age specific death rates from cancers of the "other and unspecified organs" among females, although at lower levels, display the same characteristics as the corresponding rates among males: they all rise steadily throughout the years of this experience.

Cancer Mortality by Accessibility of Site

Total accessible cancers more frequent among females.—Whereas deaths from accessible [3] cancers comprised more than 13 per cent of the recorded cancer mortality among males in 1901, accessible sites were charged with less than 7 per cent of the fatal cancers among males in 1938. While the recorded mortality for internal cancer among males more than doubled, that for accessible cancers remained practically constant. These facts point clearly to improved diagnostic technics and increased opportunities for their use, through hospitalization, operation, and autopsies, as a possible explanation of the increase in the mortality rate from cancer among males. The question then arises: how much of this increase for inaccessible sites is due to improved technics, and how much of it is possibly due to a real increase? Unfortunately, there is no way of studying this question objectively.

Among females, the mortality ascribed to cancers of accessible sites rose significantly from 41.1 per hundred thousand females in 1901 to 52.9 in 1938. But the ratio of the death rate from cancers of accessible sites to the total cancer mortality among females remained practically constant at 40 per cent. (Excluding the mortality from breast cancer, the standardized death rate from cancers of accessible sites among females rose from 27.3 in 1901 to 28.0 in 1938, an increase that is without statistical significance.) As shown in a footnote to Table III, the trend of the standardized death rates from accessible cancers among females, excluding the breast, was downward, but not significantly.

The fact that the greatest part of the increase in the cancer death rate over the past 38 years has been recorded among males, combined with the further fact that less than 7 per cent of the fatal cancers among males are external or accessible for diagnosis, lends weight to the suggestion that in the early years of this study cancer was grossly understated as a cause of death because it was not diagnosed. This leads to the further conclusion that much, if not all, of the apparent increase in cancer mortality has resulted directly from improved diagnostic technics and the increased opportunities for their use.

The fact that 40 per cent of the fatal cancers among females are in external or accessible sites, and were as readily diagnosed at the outset of our study as they are today, may explain why the recorded cancer death rate among females was so much higher in those days than the male rate and why the female rate has not been susceptible of the same rate of increase as has been recorded for males.

The fact that the proportion of external and accessible cancers among males is only one-sixth as great as among females may also explain the currently observed excess of cancer mortality among males. It is not difficult to believe that with such a high proportion of cancers located internally, the opportunities for early diagnosis and cure are lessened. From this, it also appears probable that the excess among males will be a characteristic of cancer mortality in the future.

Cancer Mortality by Sex and Age Groups

At ages below 24 years, taken as a group, the mortality ascribed to cancer is relatively low (less than 5 per hundred thousand of population) and the male rate is slightly higher than that among females. From 25 to 54, the mortality is higher among females, while from 65 to the end of life, cancer death rates are higher among males. The rates, low in the early years for both sexes, rise, without interruption, to reach their maxima in the highest age groups. The rate of increase, however, appears to have no functional relationship with age in either sex. At ages over 75 years, the cancer death rate among males is 10 times that in the age period 45 to 54 years; among females, it is 7 times as great.

It is interesting to note that there is no perceptible increase in the recorded cancer mortality among males below 35 years; that the increase is very slight in the age group 35 to 44, but that considerable increases are recorded in every age group over 45 years. The same general observations hold for females; the only change being that the curves show no important upward trend until after 55 years of age. These observations are supported by the data in Table IV.

Importance of Cancer Mortality at Various Ages—by Sex

Elsewhere (2) we have shown that in New York City in 1937, cancer was charged with 12.6 per cent of the total mortality among males and 15.3 per cent of the total deaths among females. In 1938, the mor-

[3] The classification of sites by accessibility follows that of Dr. T. H. C. Stevenson, who rendered distinguished service for so many years as the Chief Medical Officer in the office of the Registrar General of England and Wales. In general, accessible sites include the skin, breast, buccal cavity. To these are added, for females, the uterine cervix.

tality ascribed to cancer accounted for almost 14 per cent of the total male death rate and for more than 16.5 per cent of the deaths among females.

In view of the fact that the relative importance of cancer as a cause of death is dependent upon variations in the mortality from all causes and in view of the further fact that the 1938 mortality experience in the city was extremely favorable—the general death rate was 9.8 per thousand of population, the lowest ever recorded for the city—we will assume that the experience of 1937 was typical of cancer mortality in New York City during recent years. The relative importance of cancer as a cause of death in each sex at various age groups in 1937 is presented in Table V.

TABLE IV: CITY OF NEW YORK. CANCER, ALL FORMS, ALL SITES. AVERAGE SPECIFIC DEATH RATES PER 100,000 AND TREND OF MORTALITY BY SEX AND AGE, 1901 TO 1938

Age group	Averages of ages Specific death rates		Trend of recorded mortality—Average annual change (Per cent of average of rates)	
	Males	Females	Males	Females
All ages	99.3 *	113.1 *	+2.0	+0.8
0-24	4.1	3.5	+0.3 †	+0.7
25-34	12.9	23.1	+0.1 †	−0.03 †
35-44	50.2	101.3	+0.5	−0.6
45-54	182.9	266.7	+1.4	+0.05 †
55-64	482.4	518.6	+1.8	+0.7
65-74	858.3	796.9	+2.3	+1.3
75 and over	1140.7	1032.9	+3.4	+2.5

* Standardized.
† Indicates nonsignificant trend.
NOTE: For a discussion of trend values and their significance, see Note, Table II.

At all ages combined, cancer was responsible for 12.6 per cent of the mortality among males. Below 25 years, cancer was responsible for only 1.6 per cent of the mortality among males. From this minimum, the importance of cancer as a cause of deaths mounts, until it reaches a maximum in the age group 55 to 64. At this age, 17.9 per cent of all male deaths were ascribed to this cause. In the next higher decennial age period, 65 to 74, the mortality also exceeded 17 per cent. At these ages, more than one death in every six among males was charged to cancer. Although the cancer mortality rate reaches its maximum at ages over 75, because of the more rapid increase in the mortality from diseases of the cardio-vascular-renal group, the proportion of the total male deaths which is ascribed to cancer falls below 12 per cent.

Among females, cancer is charged with more than 15 per cent of deaths at all ages. Below 25 years, both the number of cancer deaths and the proportion of the total mortality which is ascribed to cancer, are lower than among males.

This situation is completely reversed in the age period 25 to 34, when cancer of the genital organs commences to take a serious toll among women. In the three decennial age groups 35 to 64, more than one in every five deaths among women in the City of New York is charged to cancer. Cancer mortality among females reaches its greatest relative importance in the age group 45 to 54, when slightly less than 25 per cent of deaths among women are charged to this cause. This is almost one death in every four.

TABLE V: CITY OF NEW YORK. IMPORTANCE OF CANCER MORTALITY BY AGE AND SEX, 1937

Age period	Males			Females		
	Deaths all causes	Deaths from cancer	Per cent cancer of total	Deaths all causes	Deaths from cancer	Per cent cancer of total
All ages	42,072	5,297	12.6	35,393	5,417	15.3
Under 25	5,042	81	1.6	4,228	56	1.3
25-34	2,140	87	4.1	2,025	154	7.6
35-44	4,478	382	8.5	2,971	626	21.1
45-54	7,450	1,007	13.5	4,741	1,109	23.4
55-64	9,344	1,668	17.9	6,870	1,445	21.0
65-74	8,449	1,462	17.3	7,869	1,315	16.7
75 and over	5,169	610	11.8	6,689	712	10.6

TABLE VI: CITY OF NEW YORK. EXPECTANCY OF DYING FROM CANCER

Age interval	Males			Females		
	Expected deaths, all causes *	Expected deaths, from cancer *	Chances of dying from cancer, per cent	Expected deaths, all causes *	Expected deaths, from cancer	Chances of dying from cancer, per cent
25+	100,000	13,844	13.8	100,000	14,684	14.7
35+	97,009	13,722	14.1	97,210	14,472	14.9
45+	90,504	13,167	14.5	92,517	13,483	14.6
55+	75,147	11,092	14.8	81,813	10,979	13.4
65+	46,771	6,026	12.9	59,973	6,385	10.6

* All of these calculations are based upon the assumption that 1937 rates will prevail during the lifetime of a hypothetical population of 100,000 at age 25.

At ages over 65 years, cancer is charged with a lower percentage of the deaths among women than among men.

CHANCES OF DYING FROM CANCER

In the same article (2) it was shown that on the basis of the recent cancer mortality experience in the City of New York, one person out of every seven who attains 25 years of age may expect to die from cancer. For completeness, the data are repeated in Table VI.

If 100,000 males, all 25 years of age could be observed until they all died, it would be found that 13,844, or 13.8 per cent of them would have died from cancer, if the mortality rates for 1937 in New

York City prevailed during the entire life span of the group. Among those who survived until the age of 35, 14.1 per cent would have died from cancer. In this hypothetical population, each subsequent decade of life involves a greater chance of dying from cancer until the peak is reached at 55, when 14.8 per cent of all living males may expect to die from this cause. Thereafter, the chances of death from cancer diminish, because other diseases of old age account for an increasing proportion of deaths.

Of 25-year-old females, 14.7 per cent may expect to die from cancer. This expectancy is initially higher among females than among males, because the mortality from cancer of the female genital organs commences to assume importance at this relatively early age. The chance of death from cancer among females reaches its highest point at 35 years, when 14.9 per cent of the survivors may expect to die from this cause. Thereafter the decline is steady, until, at ages over 65, the chance that a woman will die of cancer is only slightly greater than one in ten.

At every age from 25 until 55 years of age, females have a greater chance of dying from cancer than males; after 55, the chance that death will be due to cancer is greater among males.

Summary and Conclusions

From the data that have been presented, certain facts emerge and certain conclusions appear to be justified. Among them may be mentioned:

1. The *recorded* mortality from cancer in New York City increased steadily from 1901 to 1938.

2. The apparent rate of increase has been much greater among males than among females. The more rapid rise in the male death rate from cancer has probably been due to the fact that cancer was grossly understated in the early years, because modern technics for diagnosing internal cancers had not been developed, and because opportunities for making an accurate diagnosis were more limited than they are today.

3. In 1901, the male cancer death rate was only 68 per cent of that among females; in 1938, the standardized male death rate from cancer was 10.5 per cent higher than the female rate. Male cancer death rates have exceeded the female rates in 6 of the last 9 years, and without exception in the last 4 years.

4. With the exception of cancer of the female breast, the recorded increase in cancer mortality in both sexes has been largely limited to internal sites, normally inaccessible for diagnosis and treatment. Over the entire period of 38 years, the recorded mortality has not increased among males for cancer of the skin; among females for cancer of the buccal cavity, the stomach and liver, the genital organs, and the skin. For the past 20 years, the mortality ascribed to cancer of the stomach and liver among males has shown a downward trend, but the rate of decline is not statistically significant. The recorded mortality from cancers of the buccal cavity among males, which was less than 6 per 100,000 in 1905 was also less than 6 in 1933. From that year onward, however, there has been a steady rise.

5. In view of the fact that the increased mortality recorded has been largely limited to internal and inaccessible sites, it is possible that the higher cancer mortality rates currently observed, have resulted largely, if not entirely, from better diagnosis rather than from any real increase in cancer incidence or mortality.

6. The apparent increase in the mortality ascribed to cancer of the breast among females has been offset by a decline in the mortality ascribed to cancer of the stomach and liver. It has been suggested by others that there is a direct relationship between these two contrary trends and that they are due to more accurate reporting of the primary site on current death certificates.

7. After childhood, when the number of deaths ascribed to cancer in both sexes is low, the death rate from cancer among females exceeds that of males in age groups up to 55 years of age. Beyond 55, the male rate is higher in increasing proportion with advancing age.

8. In 1938, cancer was charged with slightly more than 15 per cent of deaths at all ages in the City of New York. Although the cancer death rate reaches its maximum at the highest ages, the relative importance of cancer as a cause of death is greatest among males in the age group 55 to 64 and among females at ages between 45 and 54.

9. If the death rates of recent years continue, one person in every 7 who reaches the age of 25 years in the City of New York may expect to die of cancer.

10. In New York City during 1938, deaths reported to be caused by cancer totaled 11,160 of which 3,987 diagnoses, or 36 per cent, were reported to have been corroborated following operation or autopsy. This percentage of verified diagnoses is probably understated. It is highly desirable, from the point of view of medical and statistical research, that more autopsies be routinely performed.

References

1. Bigelow, G. A., and H. L. Lombard. Change in the Massachusetts Cancer Trend. New England J. Med., **210**: 526-529. 1934.
2. Duffield, T. J. Cancer Mortality in New York City. Nat. Bull. Am. Soc. Control Cancer, **21**: No. 5, 4-6. 1939.
3. Duffield, T. J., and Di Mario, M. The Effect of Ageing of Population in New York City Upon the Crude Cancer Mortality Rates. Unpublished paper.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

ANDERVONT, H. B., and M. B. SHIMKIN. [Nat. Cancer Inst., Bethesda, Md.] TUMORS IN MICE INJECTED WITH COLLOIDAL THORIUM DIOXIDE. J. Nat. Cancer Inst., 1: 349-353. 1940.

Colloidal thorium dioxide injected intravenously into strain A mice in doses of from 0.2 to 0.8 cc. did not induce pulmonary tumors in these animals. Three subcutaneous tumors were induced in 9 mice, 15 to 18 months after subcutaneous injection of 0.2 cc. of colloidal thorium dioxide. Nonspecific pulmonary lesions were noted similar to those produced by the injection of finely divided quartz. Thorium dioxide particles were observed in the liver and spleen of the injected animals and there was regularly a productive fibrous splenitis.—L. L. W.

BLUMBERG, H., and R. E. GARDNER. [Sch. of Hyg. and Pub. Health, Johns Hopkins Univ., Baltimore, Md.] ADENOMATOUS STOMACH LESION OF THE RAT ASSOCIATED WITH HEAVY CYSTICERCUS FASCIOLARIS INFESTATION. Proc. Soc. Exper. Biol. & Med., 45:673-677. 1940.

Infestations with *Taenia taeniaeformis* were produced by feeding rats terminal proglottids or ova. Grossly thickened stomachs were seen in all of 7 rats, 3 males and 4 females, killed after a heavy *Cysticercus* infestation of 235 to 272 days. The enlarged livers contained 150 to 200 cysts. The lesions in the stomach were confined to the glandular portion, which was thickened and papillomatous. Microscopically the lesion appeared adenomatous. Rats with light infestations of *Cysticercus* did not develop these lesions in the stomach. In neither the heavily nor lightly infested animals was there any gross evidence of *Cysticercus* sarcomas in the liver at the time of autopsy. The adenomatous stomach lesions were produced in 3 strains of rats, piebald rats of the McCollum strain, and albinos of the Wistar and Buffalo strains. All were maintained on the McCollum stock diet without supplements.—S. B-J.

FRANSEEN, C. C., J. C. AUB, and C. L. SIMPSON. [Collis P. Huntington Memorial Hosp., Boston, Mass.] THE EXPERIMENTAL PRODUCTION OF FIBROSARCOMAS OF BONE. Cancer Research, 1:393-396. 1941.

The authors have attempted, over a period of 4 years, to produce osteogenic sarcomas by implanting methylcholanthrene, radon seeds, and thorotrast crystals near the upper tibial epiphysis and subperiosteally in rats and mice. Seventeen fibrosarcomas were produced in mice, and all were thought to be of extra-osseous origin. In 3 mice in which the methylcholanthrene had been placed in both upper tibial epiphyses, tumors developed on both sides almost simultaneously. No tumors could be produced in the Slonaker strain of albino rats.—Authors' abstract.

KLENITZKY, J. S. [Lab. of Cancer Research of the All-Union Inst. of Exper. Med., Leningrad] ON EXPERIMENTAL CANCER OF THE UTERINE CERVIX. Bull. biol. et méd. expér. URSS., 9:3-7. 1940.

Into cylindrical glass tubes 0.2 cm. in diameter and about 2 cm. long with fire-polished edges, small cotton plugs were inserted and the substance to be tested packed on the plug with a small stick. The tube was introduced into the vagina until it touched the cervix; then the plug and contiguous substance were expelled with a stylet. Administration of experimental material was made every other day, the old plug being first removed.

The effect of coal tar of proved potency was studied in this manner on the uterine cervices of 148 female white mice begun when they were 6 to 8 weeks old. Sections were made of the entire vagina and uterus from about 3 weeks on. At 3 weeks, thickening of the vaginal epithelium, especially in the vault, with hyperkeratosis, vascular dilation, and infiltration of small cells was noted. At 2 months the columnar epithelium in the cervical canal and uterus was replaced by stratified squamous epithelium; this metaplasia extended to the uterine horns. Similar changes appeared even after 24 months. Tumors, however, were not obtained.

To compare with a typical inflammatory reaction, an experiment was done with 60 female mice with plugs smeared with "formol oil." Here the response was frankly inflammatory, with insignificant atypical epithelial proliferation. The reaction to the coal tar was therefore of a different nature biologically; possibly to be considered precancerous.

In another experiment, 170 female mice each received 50 plugs with coal tar and were injected subcutaneously with 200 mouse units of folliculol in oil every 10 days throughout the life of the animal. The last survivor lived 13 months. Cancer of the uterine cervix was obtained in 2 cases (8 months and 13 months). Sections showed abundantly proliferating epithelial cells infiltrating adjacent tissues.

An experiment with 100 mice in which 0.23% "dibenzpyrene" dissolved in olive oil was applied to the cervix gave no cancer up to 13 months.—M. B.

LAW, L. W. [Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.] THE CANCER PRODUCING PROPERTIES OF AZO COMPOUNDS IN MICE. Cancer Research, 1:397-401. 1941.

The first evidence of the production of subcutaneous tumors following injections *sub cutem* of azo compounds is recorded. Thirty-five fibrosarcomas have been induced in 109 mice of the C57 black strain, using four simple azo compounds: o-aminoazotoluene, p-dimethylaminoazobenzene (butter-yellow), 4′-hydroxy,2,3′-azotoluene and 2,3′-azotoluene. Approximately 10 mgm. of the compound dissolved in olive oil was the dosage used. The most effective compound was o-aminoazotoluene. Substitution of OH for NH_2 in the azotoluene molecule did not change the incidence of induction of sarcomas but a lengthening of the latent period resulted. Removal of NH_2 from the molecule resulted in a reduction of induced sarcomas as well as a lengthening of the latent period. Butter-yellow was less effective in inducing subcutaneous tumors than the other compounds used. A total of 13 trabecular hepatomas was induced in 215 mice of the C57 black and

Dba strains. Only slight adenomatosis of the bile ducts and little or no proliferation of connective tissue occurred.—Author's abstract.

LEUCHTENBERGER, R. and G. SICHER. [Mount Sinai Hosp., New York, N. Y.] VISCERAL LESIONS FOLLOWING SINGLE SUBCUTANEOUS INJECTION OF BENZPYRENE IN MICE. Arch. Path., 31:189-203. 1941.

After a single injection of benzpyrene in mice, generalized vascular and visceral lesions were observed, such as periateritis nodosa, diffuse myocarditis, and extensive mesenchymal proliferation, which were apparently of an inflammatory nature. Particularly notable were the lesions in the liver, spleen, and lymph nodes. These lesions occurred more frequently in the benzpyrene-treated mice in which tumors failed to develop.—H. G. W.

LORENZ, E., M. B. SHIMKIN, and H. L. STEWART. [Nat. Cancer Inst. Bethesda, Md.] PREPARATIONS OF DISPERSIONS OF CARCINOGENIC HYDROCARBONS AND HORMONES WITH THE AID OF DIOCTYL ESTER OF SODIUM SULFOSUCCINATE (AEROSOL O. T.). J. Nat. Cancer Inst., 1:355-360. 1940.

Aqueous dispersions of carcinogenic substances and hormones are of value in research because the possibly active or toxic solvents usually employed are eliminated. Such dispersions are difficult to prepare, however, and are sometimes unstable. The authors have found that the superior wetting properties of the dioctyl ester of sodium sulfosuccinate (Aerosol) largely prevent flocculation of the dispersed materials. Dispersions so prepared were tested for carcinogenic or hormonal activity on mice, and were found to be fully active.—L. L. W.

PEACOCK, P. R. [Glasgow Roy. Cancer Hosp., Glasgow] BIOPHYSICAL FACTORS INFLUENCING THE ABSORPTION AND DISTRIBUTION OF BENZPYRENE, AND THEIR BEARING ON THE MECHANISM OF CARCINOGENESIS. Am. J. Cancer, 40:251-254. 1940.

The author has examined the various tissues and body fluids of animals after the intravenous injection of colloid suspensions of benzpyrene. Mechanical factors involved in the absorption of the hydrocarbon include the size of the colloid particles, the volume of the injection, and anatomical differences in the species injected. Physicochemical factors are largely dependent upon the solubility of the hydrocarbon in the various body fluids. This can be studied by the different fluorescent phenomena of native benzpyrene and its dissolved or metabolized forms. Transplanted mouse tumors did not absorb appreciable amounts of the substance.—L. L. W.

SHEAR, M. J., and H. L. STEWART [Nat. Cancer Inst., Bethesda, Md.] and A. M. SELIGMAN [Harvard Med. Sch., Boston, Mass.] STUDIES IN CARCINOGENESIS. XIII. TUMORS OF THE SPLEEN AND LIVER IN MICE FOLLOWING THE INTRODUCTION OF HYDROCARBONS INTO THESE ORGANS. J. Nat. Cancer Inst., 1:291-302. 1940.

This work confirms the experience of others that the liver and spleen are relatively refractory to the induction of tumors by the direct implantation of a hydrocarbon into the organ. Intrahepatic and intrasplenic implants of various carcinogenic hydrocarbons were made in several strains of mice, including C3H. Five of 12 C3H mice living over 7 months developed sarcomas of the spleen. Four hepatomas and 1 adenocarcinoma of the liver developed in a total of 53 mice of the C57 black, C3H, and A strains implanted intrahepatically with either 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, or 20-methylcholanthrene.—L. L. W.

SHEAR, M. J., J. LEITER, and A. PERRAULT. [Nat. Cancer Inst., Bethesda, Md.] STUDIES IN CARCINOGENESIS. XIV. 3-SUBSTITUTED AND 10-SUBSTITUTED DERIVATIVES OF 1,2-BENZANTHRACENE. J. Nat. Cancer Inst., 1:303-335. 1940.

Sixty-six 3-substituted and 10-substituted derivatives of 1,2-benzanthracene were examined for carcinogenic potency. The compounds were administered, in most instances by subcutaneous injection of the crystalline substances, to a total of about 1,300 mice of inbred strains (chiefly strain A).

Carcinogenic activity was exhibited by 24 of the 66 compounds.

The importance of meso substitution in contributing to the carcinogenic potency of derivatives of 1,2-benzanthracene, previously indicated by the activity of the 10-methyl, the 5,10-dimethyl, the 9-methyl, and the 5,9-dimethyl derivatives, was further supported by the activity shown by an additional series of potent 10-substituted derivatives of 1,2-benzanthracene. Furthermore, tumors were produced by 9-methyl-1,2,5,6-dibenzanthracene much more rapidly than by 1,2,5,6-dibenzanthracene; and 5-methyl-3,4-benzpyrene acted even more rapidly than did 3,4-benzpyrene.

High potency was exhibited by the 5-cyano-10-methyl derivative, and lesser potency by the 10-CH_2CN and the 7-cyano-10-methyl derivatives of 1,2-benzanthracene. The 5-chloro and the 7-chloro derivatives of 10-methyl-1,2-benzanthracene were also carcinogenic.

Of particular interest was the fact that a considerable number of oxygen-containing meso-substituted derivatives were potent; *viz.*, the 10-CH_2OH, the 10-$CH_2O.COCH_3$, the 10-CHO, the 10-$CH_2.COOCH_3$, the 10-OCH_3, and the 5-$CONH_2$-10-methyl derivatives of 1,2-benzanthracene, and the 5-aldehyde of 3,4-benzpyrene.—Authors' summary.

SPENCER, R. R., and M. B. MELROY. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF CARCINOGENS ON SMALL FREE-LIVING ORGANISMS. II. SURVIVAL VALUE OF METHYLCHOLANTHRENE-ADAPTED PARAMECIUM. J. Nat. Cancer Inst., 1:343-348. 1940.

Paramecia were cultured for over a year in the presence of methylcholanthrene and the noncarcinogenic dye, fluorescein. Thereafter, small numbers of organisms were transferred to a medium containing only inorganic salts. Daily population counts were made on these colonies. It was found that the methylcholanthrene-adapted organisms survived a much longer period than did the controls or those exposed to fluorescein. The biologic significance of this increased survival period is discussed.—L. L. W.

Hormones

ALLEN, E., and W. U. GARDNER. [Yale Univ. Sch. of Med., New Haven, Conn.] CANCER OF THE CERVIX OF THE UTERUS IN HYBRID MICE FOLLOWING LONG-CONTINUED ADMINISTRATION OF ESTROGEN. Cancer Research, 1:359-366. 1941.

After long treatment with large doses of estrogen, lesions and carcinoma of the uterine cervix have appeared in 15 of 24 mice (62%) and 10 of 20 mice (50%) which survived the treatment for more than 1 year. These 2

groups were hybrids of the C57 and CBA strains, inbred with regard to mammary cancer. Genetic factors involved in mammary cancer seem to have little influence as determining factors in cancer of the cervix uteri. The 2 groups mentioned above were formed to test the possible importance of maternal transmission (milk factor) in cervical cancer. Although important in mammary cancer, maternal transmission seemed to have little to do with the incidence of experimental carcinoma, except that a certain number of animals, nursed by mothers susceptible to mammary cancer, died of mammary cancer before the age at which cervical cancer appears. The high incidence of cervical cancer in these experimental groups emphasizes that estrogen is a very important factor, not merely an incidental one, in cervical carcinogenesis.—Authors' summary.

BISCHOFF, F. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] THE ACTION OF CYSTEINE OR CYANIDE UPON GONADOTROPIC EXTRACTS. J. Biol. Chem., 134:641-647. 1940.

The pituitary and urinary (prolan) gonadotropic hormones are relatively stable to cysteine and cyanide under experimental conditions which inactivate insulin and lead to disulfide reduction. Prolonged action (24 hours) and a large excess (tenfold by weight) of cyanide produce partial inactivation of both hormones, and, within the error of assay, to the same degree. Prolonged action (48 hours) and a large excess (tenfold by weight) of cysteine hydrochloride produce more than 90% inactivation of the pituitary preparation under experimental conditions which produce 65 to 85% inactivation of prolan. These results are not in accordance with the view that the disulfide linkage is a component of the active group in the pituitary hormone but not in the urinary hormone.—Author's abstract.

BISCHOFF, F. [Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.] NON-SPECIFIC AUGMENTATION WITH A HIGHLY PURIFIED FOLLICLE STIMULATING HORMONE FRACTION (EVANS). Endocrinology, 27:554-558. 1940.

An unfractionated pituitary gonadotropic hormone preparation was not appreciably adsorbed by added $Zn(OH)_2$ and did not manifest nonspecific augmentation in the presence of such added $Zn(OH)_2$. The hormone is quantitatively precipitated by first adding the zinc as the sulfate and then precipitating the zinc as hydroxide. Under these conditions marked nonspecific augmentation is produced. A highly purified follicle-stimulating hormone fraction, prepared in the laboratory of H. M. Evans, produced augmentation (150 to 550%) when administered with zinc or copper under described conditions. This follicle-stimulating preparation when injected intraperitoneally with a simultaneous subcutaneous injection of the same preparation in copper combination, gave rise to the antagonist phenomenon; *viz.*, the preparation, by a rapid resorption rate, was able to antagonize its own influence when acting under a delayed resorption rate. In spite of all efforts at purification, the multiple hormone effects can still be produced by the so-called follicle-stimulating hormone fraction.—Author's abstract.

BLUMENTHAL, H. T. [Washington Univ. Sch. of Med., St. Louis, Mo.] THE EFFECT OF FRESH AND EXPERIMENTALLY MODIFIED ANTERIOR HYPOPHYSIS OF CATTLE ON MITOTIC ACTIVITY IN THE ADRENAL CORTEX OF THE GUINEA PIG. Endocrinology, 27:486-493. 1940.

Implantation of fresh cattle anterior hypophyseal lobes or injections of extracts of this gland cause an increase in the number of mitoses in the adrenal cortex of immature female guinea pigs. It is possible to modify this proliferating effect by subjecting the hypophyseal glands to various chemical treatments previous to implantation.—Author's abstract.

BLUMENTHAL, H. T. [Washington Univ. Sch. of Med., St. Louis, Mo.] THE INFLUENCE OF TIME OF FEEDING ON THE PERIODICITY IN ACTIVITY IN THYROID AND ADRENAL GLAND OF NORMAL MALE GUINEA PIGS. Endocrinology, 27:481-485. 1940.

Cyclic variations in the average number of mitoses in different tissues of plants and animals, corresponding to the 24-hour daily cycle, are usually explained by variations in light intensity. Our investigations indicate that, if guinea pigs are fed once in 24 hours, a rise in the proliferative activity of adrenal cortex and thyroid takes place, which as a rule reaches a maximum between 4 and 12 hours after feeding. It is probable that metabolic processes initiated by the intake of food determine this rhythmic mitotic activity.—Author's abstract.

BLUMENTHAL, H. T. [Washington Univ. Sch. of Med., St. Louis, Mo.] THE MITOTIC COUNT IN THE ADRENAL CORTEX OF NORMAL GUINEA PIGS. Endocrinology, 27:477-480. 1940.

The average number of mitoses in a certain volume of the adrenal gland of the guinea pig has been determined. It is probable that the mitotic proliferation decreases gradually from embryonic life to the age of 2 years. No significant difference in mitotic counts was observed in male and female guinea pigs. In some cases the count was higher in animals kept at a temperature of 20° C. than in those kept at 30° C.—Author's abstract.

GARDNER, W. U. [Yale Univ. Sch. of Med., New Haven, Conn.] THE EFFECT OF ESTROGEN ON THE INCIDENCE OF MAMMARY AND PITUITARY TUMORS IN HYBRID MICE. Cancer Research, 1:345-358. 1941.

Mice of a mammary tumor-susceptible strain (CBA) were mated to mice of a mammary tumor-resistant strain (C57). Seventy per cent of the females of the former strain and none of the females of the latter strain developed mammary adenocarcinomas. Fifty-two hybrid mice with CBA mothers and 53 hybrids with C57 mothers received weekly injections of 16.6 or 50 µgm. of estradiol benzoate weekly throughout life. Other mice of similar origin were untreated but permitted to breed, the young being removed at birth.

Mammary cancers appeared in 59 per cent of the estrogen-treated male and female mice with CBA mothers and in 85 per cent of the breeding hybrid females of similar origin. None of the estrogen-treated hybrid mice with C57 mothers developed mammary tumors, although 43 per cent of the breeding females had mammary tumors at death. Only about one-third of the tumors in the latter group were considered malignant. The environment of mammary glands in females subjected to continuously repeated pregnancies predisposed to a higher incidence of mammary tumors than the environment created by administration of estrogen.

Nonmalignant and premalignant localized mammary growths appeared in the estrogen-treated hybrid mice with the tumor-susceptible CBA maternal parents, but were lacking in the mice with C57 mothers. The tendency for localized overgrowths was acquired with the tendency to develop a high incidence of mammary tumors.

Hypophysial tumors, which had appeared previously only in estrogen-treated mice of the C57 strain in the laboratory, appeared in the estrogen-treated hybrids of both groups. The incidence was greater in the males than in the females. The transmission of the tendency to develop such tumors is compatible with a genetic interpretation.

The incidence of lymphoid tumors was greater in the estrogen-treated hybrids than in the controls. The adrenal glands of the mice revealed no histological changes that could be associated with the tendency to develop mammary tumors.—Author's summary.

LIPSCHÜTZ, A., P. BELLOLIO, J. CHAUME, and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] COMPARATIVE CONJUNCTIVE TUMORIGENIC ACTION OF THREE DIFFERENT ESTERS OF ESTRADIOL. Proc. Soc. Exper. Biol. & Med., 46:164-167. 1941.

When 40 subcutaneous injections of 5 to 10γ of an esterified estradiol (monobenzoic, dipropionic, or monocaprylic ester) or a total of 200 to 400γ are given to castrate female guinea pigs in the course of 3 months, uterine and extra-uterine fibroids will appear. The number of animals in which fibroids are induced is larger with the monocaprylic ester; the size of the fibroids is also much more remarkable with the monocaprylate.—S. B-J.

LIPSCHÜTZ, A., E. EGANA, I. SZABO, and S. LECANNELIER. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] ZOOLOGICAL SPECIFICITY OF THE TUMORAL REACTION TOWARDS ESTROGENS IN RATS. Proc. Soc. Exper. Biol. & Med., 46:161-164. 1941.

A quantity of the monocaprylate of α-estradiol up to 40 times that which is able to induce abdominal fibroids in the guinea pig, is insufficient to elicit similar tumors in the rat. The same or smaller quantity induces in the rat a highly pronounced atypical proliferation of epithelial tissues (endometrium, anterior lobe of the hypophysis).—Authors' summary.

LIPSCHÜTZ, A., R. IGLESIAS, and L. VARGAS, JR. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] UTERINE AND EXTRAUTERINE LOCALIZATIONS OF EXPERIMENTAL FIBROIDS INDUCED IN GUINEA PIG BY PROLONGED ADMINISTRATION OF ESTROGENS. Proc. Soc. Exper. Biol. & Med., 45:788-792. 1940.

Fibroid tumors in a number of locations in the abdominal cavity were induced in guinea pigs by prolonged administration of 10 estrogens, namely: estrone, estrol, estradiol, 4 esters of estradiol (monobenzoate, monocaprylate, dipropionate, 17-benzoate-3-n-butyrate), stilbestrol, and hexestrol. These subserous and intramural fibroids were produced in castrated female and male guinea pigs by injections of estrogens. The amounts injected, periods of treatment, and other details are given in the explanation of illustrations.—S. B-J.

LIPSCHÜTZ, A., L. VARGAS, JR., H. BAEZA-ROSALES, and H. BAEZA-HERRERA. [Dept. of Exper. Med., Nat. Health Service, Santiago, Chile] THE 17-MONOCAPRYLIC ESTER OF ESTRADIOL—A HIGHLY ACTIVE TUMORIGENIC ESTROGEN. Proc. Soc. Exper. Biol. & Med., 46:76-78. 9141.

The conjunctive tumorigenic action of α-estradiol is greatly enhanced by esterification with caprylic acid at position 17. Under certain conditions of timing of injections subcutaneously into castrated female guinea pigs, the 17-monocaprylic ester becomes 100 to 200 times as active as α-estradiol in producing fibroid tumors. With 38 injections of the 17-caprylate of estradiol given in the course of 3 months and equivalent to a total of 76γ of estradiol, typical experimental abdominal fibroids were induced. The distribution and structure of the fibroids are shown in illustrations.—S. B-J.

MENKIN, V. [Harvard Univ. Med. Sch., Boston, Mass.] EFFECT OF ADRENAL CORTEX EXTRACT ON CAPILLARY PERMEABILITY. Am. J. Physiol., 129:691-697. 1940.

An inflammatory exudate induces an increase in capillary permeability. This is manifested by the accumulation of trypan blue from the circulation in a cutaneous area previously injected with an exudate. This effect is likewise duplicated by leukotaxine, a nitrogenous substance extracted from inflammatory exudates. The effect of an exudate or of leukotaxine of increasing capillary permeability is wholly or in part inhibited by the presence of an extract of the adrenal cortex. This inhibitory effect may be brought about by adding the hormonal preparation to the exudative material and then injecting the mixture into the cutaneous tissue of a normal rabbit. Similar results can be attained by separate injections, several minutes to hours apart and at the same site, of the hormone followed by either the exudative material or leukotaxine. The apparent property of the hormone of the adrenal cortex to influence capillary filtration and therefore its possible significance in various conditions such as traumatic or secondary shock is to be noted. The practical and relatively simple technical procedure utilized in the series of experiments suggests the possibility of a convenient method for assaying various extracts of the adrenal cortex.—Author's abstract.

PERLOFF, W. H., and R. KURZROK. [Sch. of Med., Univ. of Pennsylvania, Philadelphia, Penn. and Coll. of Physicians and Surgeons, New York, N. Y.] PRODUCTION OF UTERINE TUMORS IN THE GUINEA PIG BY LOCAL IMPLANTATION OF ESTROGEN PELLETS. Proc. Soc. Exper. Biol. & Med. 46:262-265. 1941.

Pellets of estradiol benzoate each about 3 mgm. were implanted in the uterine horn of each of 12 adult virgin female guinea pigs. This was done by raising the peritoneum from the uterine muscle and placing the pellet into the pocket thus formed. A black silk suture closed the pocket and acted as marker. In 6 of these animals small paraffin or cork pellets were similarly introduced into the opposite horn. These animals were sacrificed at from 32 to 150 days. In 3 other guinea pigs a black silk suture was used to close empty pockets and an injection of estradiol benzoate of 10,000 R. U. was given subcutaneously every 20 days for a total of 60,000 R. U. In 2 additional animals 3 mgm. pellets of estrone were implanted in the uterus; and in 5 other pigs 3 mgm. pellets of testosterone propionate. Finally 2 animals each received a pellet of estradiol benzoate in one horn and a pellet of

testosterone propionate in the other. All these animals were sacrificed after 150 days.

Seven unoperated controls were normal. In the 12 guinea pigs receiving estradiol benzoate pellets into one horn of the uterus, either alone or with a cork or paraffin control in the other horn, fibromyomatous tumors were found surrounding the estrogenic pellet in 9 cases. No tumors were found around the cork or paraffin implants. Hydrometra (with or without hydrosalpinx) and pyometra (with or without hydrometra) were observed.

The 3 guinea pigs injected with estradiol benzoate subcutaneously and in which silk sutures were introduced into the uterine peritoneum showed no tumors. The 2 animals with estrone pellets showed no tumors; but the 2 animals carrying both estrogenic and androgenic pellets showed tumors only at the site of the estrone pellets.

Microscopically, the firm gray tumors were composed of varying amounts of muscular, fibrous, and collagenous tissues. The endometrial glands were cystic with widespread degeneration and necrosis. The cervical epithelium was hyperplastic with increased keratinization, downgrowth into the submucosa and pearl formation. The vagina showed marked cornification, thickening of the prickle cell layer, penetration of epithelial cords into the submucosa, and pearl formation.—M. B.

SHIMKIN, M. B., and H. G. GRADY. [Nat. Cancer Inst., Bethesda, Md.] MAMMARY CARCINOMAS IN MICE FOLLOWING ORAL ADMINISTRATION OF STILBESTROL. Proc. Soc. Exper. Biol. & Med., 45:246-248. 1940.

Stilbestrol dissolved in sesame oil was fed to 41 male C3H mice by means of a curved metal tube passed into the esophagus. Mammary cancer does not occur in males of this strain. The mice were about 2 months old and weighed 21 to 23 gm. at the start of the experiment. Animals in 4 groups were treated as follows: (I) 11 were given 0.125 mgm. stilbestrol dissolved in 0.1 cc. sesame oil twice a week for 17 weeks, total amount 4.25 mgm.; (II) 10 received 0.375 mgm. stilbestrol in 0.1 cc. sesame oil twice a week for 17 weeks, total amount 12.75 mgm.; (III) 10 were fed 0.1 cc. sesame oil twice a week for 17 weeks; (IV) 10 were kept as untreated controls. Breast tumors were recognized by palpation 20 weeks after the first feeding of stilbestrol. Of 12 treated mice alive in group I at 20 weeks, 4 developed tumors at 23, 24, 24, and 25 weeks. Of 5 in group II alive at 20 weeks, 3 developed tumors in 20, 24, and 26 weeks. All 7 tumors were adenocarcinomas. Other pathological changes in the treated mice are described.—S. B-J.

SILBERBERG, M. and R. SILBERBERG. [Oscar Johnson Inst., St. Louis, Mo.] THE EFFECT OF THYROIDECTOMY AND ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE ON THE GROWTH OF CARTILEGE AND BONE OF IMMATURE GUINEA PIGS. Am. J. Path., 16:505-524. 1940.

Thyroidectomy in guinea pigs causes an accelerated sclerosis of the cartilaginous ground substance and inhibits the complete differentiation of columnar cartilage cells into hypertrophic cartilage cells in the epiphyseal disc. Proliferation of the cartilage cells is maintained and may even be increased temporarily. Endochondral ossification is secondarily affected. Pituitary extract of cattle, injected into thyroidectomized guinea pigs, exerts its characteristic influence on cartilage and bone. Increased growth and retrogressive changes in the cartilage are followed by an enhanced ossification, but differentiation of the cartilage remains incomplete.—Authors' abstract.

SILBERBERG, M. and R. SILBERBERG. [Oscar Johnson Inst., St. Louis, Mo.] EFFECTS OF OVARIECTOMY AND LONG-CONTINUED ADMINISTRATION OF ANTERIOR PITUITARY EXTRACT OF CATTLE ON SKELETAL TISSUES OF IMMATURE GUINEA PIGS. Am. J. Path., 16:491-504. 1940.

During the first month after ovariectomy there is in immature guinea pigs an increased proliferation of the euhyaline cartilage of the epiphyseal discs and of the covering of joints and ribs, whereas calcification and ossification of joints and ribs are relatively retarded. Later, the growth-promoting effect of ovariectomy diminishes or ceases, while sclerosis of the cartilage is greatly intensified and ossification progresses. Gigantism does not occur. Injection of pituitary extract of cattle partly counteracts the effects of ovariectomy.—Authors' abstract.

SILBERBERG, M. and R. SILBERBERG. [Oscar Johnson Inst., St. Louis, Mo.] THE RESPONSE OF CARTILAGE AND BONE OF THE NEWBORN GUINEA PIG TO STIMULATION BY VARIOUS HORMONES (ANTERIOR HYPOPHYSEAL EXTRACT, ESTROGEN, THYROXIN). Anat. Rec., 78:549-558. 1940.

In newborn guinea pigs the cartilage tends to respond to various hormonal stimuli with more proliferative and less retrogressive changes than the cartilage of older animals. This is further evidence for the conclusion that the age of the animal plays an important role in determining the mode of reaction of the cartilage to the administration of hormones.—Authors' abstract.

TWOMBLY, G. H. [Memorial Hosp., New York, N. Y.] BREAST CANCER PRODUCED IN MALE MICE OF THE C57 (BLACK) STRAIN OF LITTLE. Proc. Soc. Exper. Biol. & Med., 44:617-618. 1940.

Nine male mice of this strain, in which spontaneous breast cancer has not been reported, developed breast cancer after being nursed by "high cancer" mothers (Paris III) and having estrone crystals (0.07 to 0.18 mgm.) implanted 10 days after birth.—Author's abstract.

WILSON, J. G., W. C. YOUNG, and J. B. HAMILTON. [Yale Univ. Sch. of Med., New Haven, Conn.] A TECHNIC SUPPRESSING DEVELOPMENT OF REPRODUCTIVE FUNCTION AND SENSITIVITY TO ESTROGEN IN THE FEMALE RAT. Yale J. Biol. & Med., 13:189-202. 1940.

This technic permits the preparation of female animals whose reproductive cycle fails to appear. Further, the animals remain insensitive, apparently permanently, to estrogens. This insensitivity was determined morphologically by uterine histology, physiologically by failure of estrual behavior. Tests are now in progress to determine the response to estrogens of similarly prepared mice with mammary and uterine tumors.—Authors' abstract.

WOLFE, J. M., E. BURACK, W. LANSING, and A. W. WRIGHT. [Albany Med. Coll., Albany, N. Y.] CHANGES IN THE CONNECTIVE TISSUE OF THE UTERUS AND VAGINA OF THE RAT ASSOCIATED WITH ADVANCING AGE. Science, 91:577-578. 1940.

Loeb and associates found that, with advancing age, there is an increase in the amount of fibrillar and hyaline connective tissue in the uterus, vagina, and cervix of the mouse. Using methods which differentiate reticulum from collagen, the authors have carried out similar studies in

the rat. Briefly, the uterine connective tissue in 15-day rats is chiefly reticulum. As age advances this reticular tissue changes into collagen in a more or less characteristic fashion. These changes are described. The studies support the view that reticulum is a precollagenous type of connective tissue and may be transformed into collagen—Authors' abstract.

WOODRUFF, L. M. [Yale Univ. Sch. of Med., New Haven, Conn.] TUMORS PRODUCED BY ESTRADIOL BENZOATE IN THE GUINEA PIG. Cancer Research, 1:367-370. 1941.

Estradiol benzoate pellets were inserted subcutaneously in female guinea pigs whose uterus and ovaries had been removed, and in normal male guinea pigs. Those animals receiving 50 mgm. pellets developed tumors on the peritoneum in all cases. These tumors varied in composition, some being fibromas, others leiomyofibromas. The serosal cells adjacent to and covering the tumors were hypertrophied and hyperplastic, in many instances appearing to take part in the formation of the tumors. Ducts and pseudo-acinar structures within the tumors were lined by hypertrophied cells which were continuous with the serosal covering cells. Small abscess pockets found about the vaginal cuff in 3 instances were surrounded by a great thickness of granulation tissue, which, at its periphery resembled the tumor nodules microscopically.—Author's abstract.

Viruses

DURAN-REYNALS, F., and E. ESTRADA. [Yale Univ. Sch. of Med., New Haven, Conn.] PROTECTION OF CHICK AGAINST ROUS SARCOMA VIRUS BY SERUM FROM ADULT CHICKENS. Proc. Soc. Exper. Biol. & Med., 45:367-372. 1940.

Newly hatched Plymouth Rock chicks were first injected intraperitoneally with 0.5 cc. serum from pooled serum from 10 Plymouth Rocks 8 months old. As controls an equal number of chicks was similarly injected with either saline solution or rabbit serum. Next day the chicks were injected in the jugular vein with 0.5 cc. of Berkefeld N filtrate of Rous tumor extracted with 19 parts saline. The peritoneal injections of serums or saline were repeated daily until the first chick died, usually 8 to 12 days after the injection; then the rest of the animals were killed for study. If no animals had died by the 12th day, all in that group were killed. Out of 12 experiments protection against the effect of the virus by treatment with chicken serum was obtained in 9. None of 78 treated animals died in 12 days whereas 17 of 80 controls died in this time. The incidence of lesions around the injected vein, generalized hemorrhagic lesions, generalized neoplastic lesions, or combined lesions, and blood clots in the peritoneum was considerably less in the animals treated with chicken serum than in the controls. The results showed that the adequate treatment of highly susceptible chicks by serum from chickens caused either a shift from hemorrhagic into neoplastic lesions or suppression of all lesions. This protective action against the Rous virus is attributed to the antibody-like factor present in the serum of adult chickens.—S. B-J.

FRIEDENWALD, W. F., and R. S. ANDERSON. [Rockefeller Inst. for Med. Research and Memorial Hosp., New York, N. Y.] FACTORS INFLUENCING THE INACTIVATION OF THE RABBIT PAPILLOMA VIRUS BY X-RAYS. Proc. Soc. Exper. Biol. & Med., 45:713-715. 1940.

Virus was obtained by extracting rabbit papilloma tissue in 10 to 20 volumes of isotonic saline solution, centrifuging at 4,400 r.p.m. for 20 minutes and filtration through Berkefeld V candles. Partially purified suspensions of the virus were prepared by centrifuging the filtrate at 30,000 r.p.m. for 1 hour. The small pellet was resuspended in isotonic saline to the original volume and centrifuged at 4,400 r.p.m. for 20 minutes. These 2 steps in centrifugation were then repeated. The final virus suspensions were faintly opalescent and contained less than 1% as much nitrogen as the whole filtrate. Twenty to 30 cc. of the virus suspension were irradiated in discoid Pyrex flasks, 1.8 cm. deep with thin flat top and bottom. Flasks were placed between 2 x-ray tubes, 10 cm. from each target. Temperature did not rise above 30° C. The x-rays were not filtered. The x-ray tubes were run at 30 ma. and 180 to 200 kv., giving 6,200 r.p.m. and the half-layer of radiation was 0.19 mm. copper. Small samples of virus were removed at intervals and tested for infectiousness by rubbing them into freshly scarified skin of domestic rabbits. Other suspensions of virus were mixed with 10% normal rabbit serum and irradiated. Ten experiments were carried out with consistent results.

Only a small portion of virus in whole Berkefeld filtrates remained active after 1,000,000 r, and 2,000,000 to 4,000,000 r made the filtrates completely innocuous. Saline suspensions of partially purified virus were almost completely inactivated by 100,000 r; completely by 500,000 to 800,000 r. The amount of radiation required to inactivate was proportional to the amount of virus in the suspension. Suspensions of virus in 10% normal rabbit serum required more irradiation for inactivation than suspensions in saline solution. It seems probable that the protection of papilloma virus from x-rays by normal serum or by extrinsic material present in papilloma extracts is attributable to extraneous protein.—S. B-J.

Genetics

BARRETT, M. K. [Nat. Cancer Inst., Bethesda, Md.] THE INFLUENCE OF GENETIC CONSTITUTION UPON THE INDUCTION OF RESISTANCE TO TRANSPLANTABLE MOUSE TUMORS. J. Nat. Cancer Inst., 1:387-393. 1940.

Mice of the Rockefeller Institute, Strong A, and C57 black strains were given 0.2 cc. of defibrinated mouse blood from the 3 strains. Ten days later they received tumor grafts of one of three transplantable mammary carcinomas. These were (1) Bashford No. 63 (genetically indifferent); (2) No. 15091-A (origin in A mouse, but transplantable outside the parent strain); and (3) No. 775 (origin in C57 black). Controls received the graft alone. It was found that wide differences in susceptibility to the implanted tumors occurred. Generally, when the injected blood was closely related to the tumor but not to the host, a high degree of resistance was obtained. No significant resistance could be induced against a homologous tumor by either homologous or heterologous blood.—L. L. W.

DeOME, K. B. [Univ. of California, Berkeley, Calif.] THE INCIDENCE OF MAMMARY TUMORS AMONG LOW-TUMOR STRAIN C57 BLK MICE WHEN FOSTER-NURSED BY HIGH-TUMOR STRAIN A FEMALES. Am. J. Cancer, 40:231-234. 1940.

This paper adds confirmation to Bittner's work on the effect of foster nursing upon tumor incidence in inbred mice. The mammary tumor incidence of resistant C57 BLK breeding females was raised from below 1% to

20.9% by suckling these mice on lactating strain A females. The numbers of mice used were statistically significant.—L. L. W.

Radiation

BLUM, H. F. [Nat. Cancer Inst., Bethesda, Md.] SUNLIGHT AND CANCER OF THE SKIN. J. Nat. Cancer Inst., 1:397-421. 1940.

In this review the author has assembled numerous interesting facts relative to light and its effect on cutaneous tissues. He concludes that there is much evidence that sunlight of wave lengths shorter than 3,200 A. is an etiologic factor in the production of skin cancer, but admits that "much more exact data both clinical and experimental are required to place the concept on a thoroughly sound footing." A large bibliography is appended. —L. L. W.

HENSHAW, P. S. [Nat. Cancer Inst., Bethesda, Md.] RADIATION AND THE CELL. J. Nat. Cancer Inst., 1: 277-290. 1940.

After explaining the effects of wave and particle radiation on atoms, the author examines the dose-action curves of radiation on varied biologic materials. From a study of these he concludes that free living cells at least may respond to radiation in three patterns which may be construed: (1) as a result of single-event action, (2) as a result of multiple-event action, effect manifest in holistic fashion, and (3) as a result of multiple-event action, effect manifest by degree. The response of various cells seems to indicate that isolated events, even the production of single ion pairs, may bring about far-reaching biological changes. If this is true and the entities that control the destiny of the cell are of molecular size or smaller, then the importance of the irradiation technic in studying the economy of the cell is obvious.—L. L. W.

HUEPER, W. C. [Warner Inst. for Therapeutic Research, New York, N. Y.] CUTANEOUS NEOPLASTIC RESPONSES ELICITED IN HAIRLESS RATS AND IN THEIR HAIRED LITTER MATES BY ULTRAVIOLET RAYS. Cancer Research, 1:402-406. 1941.

When hereditarily hypotrichotic rats and their haired litter mates were exposed to ultraviolet rays from a mercury vapor lamp, the animals of the haired series developed multiple carcinomas and sarcomas, while the animals of the hairless series reacted with an accentuation of the normal hyperkeratotic conditions and with the appearance of a moderate number of cornified horns. Only one of the hairless rats developed carcinoma of the skin. This difference in susceptibility to actinic carcinogenesis is attributed to the presence of a normally thickened and keratotic skin in the hairless animals which offer for this reason a greater resistance to the ultraviolet rays. The frequent coexistence of neoplastic and arteriosclerotic and arteriocalcinotic changes in the irradiated rats suggests that both manifestations are the results of an excessive exposure to the actinic energy. It is uncertain whether the same photochemically generated agent is responsible for their production. Inasmuch as the two reactions are appreciably more frequent and more severe among the haired animals, having an average life span of 555 days, than among the hairless rats, possessing an average life span of 360 days, it does not seem probable that physiological aging processes play any significant role in the production of the cancerous and arteriosclerotic lesions in the irradiated rats.—Author's abstract.

JONES, H. B., I. L. CHAIKOFF, and J. H. LAWRENCE. [Univ. of California, San Francisco, Calif.] PHOSPHORUS METABOLISM OF THE SOFT TISSUES OF THE NORMAL MOUSE AS INDICATED BY RADIOACTIVE PHOSPHORUS. Am. J. Cancer, 40:235-242. 1940.

Strain A mice were injected intraperitoneally with 0.2 cc. of radioactive sodium phosphate containing 3 mgm. of phosphorus per cc. and 5 microcuries of radioactivity per 0.2 cc. The animals were sacrificed at intervals thereafter and the content of labeled phosphorus in the various tissues measured. All the tissues except the brain showed a sharp early rise in concentration of phosphorus, the maximum deposit being between 5 and 10 hours. Thereafter, there was a progressive fall. The brain, in contrast, showed a progressive rise in concentration of P^{32}. Calculated as deposit or removal per gm. of tissue, the labeled phosphorus was most actively metabolized by liver, small intestine, and kidney; next in order were muscle, lymph nodes, and lungs. The lowest activity was found in blood and brain.—L. L. W.

JONES, H. B., I. L. CHAIKOFF, and J. H. LAWRENCE. [Univ. of California, San Francisco, Calif.] PHOSPHORUS METABOLISM OF NEOPLASTIC TISSUES (MAMMARY CARCINOMA, LYMPHOMA, LYMPHOSARCOMA) AS INDICATED BY RADIOACTIVE PHOSPHORUS. Am. J. Cancer, 40:243-250. 1940.

The content of radioactive phosphorus in 3 transplantable tumors (mammary carcinoma, lymphoma, and lymphosarcoma) was measured at intervals following intraperitoneal injection of the isotope into strain A mice in which the tumors were growing. Double or triple implants in each mouse were studied. The tumor tissues show a high and rapid uptake of labeled phosphorus similar to the most active normal tissues. They retain this phosphorus for long periods which normal tissues do not do. In general, the trends of deposition and removal of the labeled phosphorus by the 3 tumors are similar. In spite of the dissimilarity of their phospholipid activities, lymphoma and lymphosarcoma took up P^{32} at about the same rate. The phosphorus activity of the lymphoid tumors was at least twice as great as that of normal lymph nodes.—L. L. W.

OSGOOD, E. E. [Univ. of Oregon Med. Sch., Portland, Ore.] EFFECTS OF IRRADIATION ON LEUKEMIC CELLS IN MARROW CULTURES. Proc. Soc. Exper. Biol. & Med., 45:131-135. 1940.

The marrow culture technic was used to study the effect of x-rays on living normal and leukemic cells from man. After cell counts were made, 50 cc. of culture containing about 100,000,000 nucleated cells in a medium consisting of 65% balanced salt solution and 35% human cord serum was mixed and 8 cc. portions placed in 30 cc. vaccine vials. One vial was used as control; the others were given various doses of radiation at 200 kv., 7 ma., 1 mm. copper and 1 mm. aluminum, at 40 cm. distance, 22 r per minute and 0.063 A minimum wave length, and a half value layer of 1.54 mm. copper. Total and differential counts were repeated at intervals up to 96 hours of incubation of cultures after irradiation. In irradiated cultures of nonleukemic cells the ratio of effect of 50 r on progranulocytes to the effect on more susceptible lymphocytes was the same as

with a dose of 400 r. An increase of 8 times the amount of irradiation, from 50 to 400 r, increased the effect on either progranulocytes or lymphocytes only 1.7 times. Leukemic progranulocytes showed a greater decrease from 300 r than nonleukemic progranulocytes from a dose of 400 r. The mechanism of action of irradiation on leukemic cells is probably a prevention of the onset of cell division.—S. B-J.

Biochemistry and Nutrition—Chemotherapy

ANDERVONT, H. B. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF COLCHICINE AND BACTERIAL PRODUCTS ON TRANSPLANTABLE AND SPONTANEOUS TUMORS IN MICE. J. Nat. Cancer Inst., 1:361-366. 1940.

Mice of the L, A, and ABC strains with transplanted tumors (sarcoma 37 or carcinoma F) and C3H mice with spontaneous mammary carcinomas were injected intraperitoneally with colchicine in saline or a filtrate of *Bacillus coli*. In some of the experiments the animals were injected with both the colchicine and the filtrate. It was found that 0.1 mgm. colchicine and 0.1 cc. of the undiluted filtrate of a week-old broth culture of *B. coli* were nearly equally effective in producing hemorrhages in the various tumors. Above 0.1 mgm., the colchicine was definitely toxic. Small repeated doses failed to influence the growth of the tumors in any way, while repeated doses each large enough to produce hemorrhages killed the animals. Given together, the colchicine and bacterial filtrate supplemented each other but the hemorrhagic effect on the tumors was not greater than that produced by either substance. Carcinoma F, which was at first resistant to the action of bacterial filtrate became susceptible after the 17th passage. —L. L. W.

ANDO, T. [Path. Inst. der Kais. Univ. zu Kyoto] EINFLUSS DER FUTTERMITTEL AUF DIE EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG. (II MITTEILUNG). EXPERIMENTELLE LEBERKARZINOMENTSTEHUNG UND GETREIDE. (ZEITDAUER DER FÜTTERUNG: ÜBER 280 TAGE.) [INFLUENCE OF DIET ON EXPERIMENTAL LIVER CARCINOGENESIS (COMMUNICATION II). EXPERIMENTAL LIVER CARCINOGENESIS AND GRAINS. (DURATION OF FEEDING: OVER 280 DAYS).] Gann, 35:50-52. 1941.

In a previous communication it was reported that after 280 days of feeding o-aminoazotoluene mixed with various grain diets, wheat protected completely against hepatic carcinogenesis, unpolished rice was less effective, while polished rice was least effective unless supplemented with yeast. In continuation of this study, 3 groups of rats were observed on the following diets containing 5% o-aminoazotoluene in olive oil (27 gm./kg. in diet I, and 20 gm./kg. in diets II and III): I. wheat (5 rats); II. unpolished rice (3 rats); and III. polished rice+yeast (1 rat). Hepatomas developed in 3 rats on diet I after 403, 505, and 550 days; in 2 rats on diet II after 488 and 545 days; and in the one rat on diet III after 513 days. The author concludes that while a wheat diet protects against hepatic carcinogenesis by o-aminoazotoluene up to 280 days, this protection does not persist. Rats on a wheat diet with and without o-aminoazotoluene grew better than rats on other diets.—P. P. C.

BRUNSCHWIG, A., and R. A. RASMUSSEN. [Chicago Univ. Sch. of Med., Chicago, Ill.] THE RELATION OF DIET TO BENIGN NEOPLASIA (ULCERO-PAPILLOMAS) OF THE RAT'S STOMACH. Cancer Research, 1:371-378. 1941.

A number of investigators have reported the development of ulcero-papillomas in the rumen and glandular portions of the stomach in rats receiving various types of deficient diets. The explanations for the appearance of such lesions have been given by them as vitamin A deficiency, vitamin B deficiency, protein starvation, infection by *Spiroptera,* carcinogenic action of heated lard, etc.

The authors conducted experiments using white rats of a colony inbred for 8 years in which various groups were fed deficient high fat, high carbohydrate and high protein diets, with and without adequate quantities of vitamins A, B, C, and D. The development of ulcero-papillomas in the gastric mucosa was observed in animals receiving the various diets. It appeared that no one specific type of deficient diet was the principal etiologic factor. The lesions were not due to heated fats in some of the diets. Emaciation *per se* was not the etiologic factor. It was concluded that the lesions might be a nonspecific reaction of the rat's stomach to a certain degree of malnutrition resulting from any type of deficient diet. Pure protein diet (casein), but not necessarily a high protein diet (50%) afforded protection against the lesions. Absolute starvation, except for water, was endured for only 1 to 7 days, the animals dying after marked loss of weight and exhibiting acute hemorrhagic erosions of the gastric mucosa. The latter were interpreted as a different order of reactions from the development of the proliferative lesions. The ulcero-papillomas once developed as a result of deficient diet, disappeared after approximately 2 weeks on an adequate well balanced diet.—Authors' abstract.

CROOKE, A. C. and R. K. CALLOW. [The Med. Unit of the London Hosp. and the Nat. Inst. for Med. Research, London] THE DIFFERENTIAL DIAGNOSIS OF FORMS OF BASOPHILISM (CUSHING'S SYNDROME) PARTICULARLY BY THE ESTIMATION OF URINARY ANDROGENS. Quart. J. Med., 8:233-249. 1939.

Four cases presenting a clinical picture of Cushing's syndrome were described. One male and 1 female had tumors of the adrenal cortex and 2 females had no cortical tumors. Urines were obtained from the 2 patients with cortical tumors and of 1 of the latter patients. The urines of the 2 patients gave high values in the colorimetric assay of 17-ketosteroids or in biological assay of androgens. Transdehydroandrosterone was isolated in amounts 2 to 35 times greater than those occuring in normal men and women. The determination of 17-ketosteroids was discussed as a method for the determination of adrenal cortical tumors in basophilism. Photographs of the patients and 1 x-ray film are included.—W. U. G.

DORFMAN, R. I., and J. B. HAMILTON. [Yale Univ. Sch. of Med., New Haven, Conn.] CONCERNING THE METABOLISM OF TESTOSTERONE TO ANDROSTERONE. J. Biol. Chem., 133:753-760. 1940.

Preceding studies indicated certain possible intermediary substances in the metabolism of androgens. Three possibly intermediate substances were synthesized and administered to eunuchs and to eunuchoid men to test inactivation pathways. The urine was examined for steroid end products. After administration of these and other androgenic steroids (dehydroisoandrosterone, androsterone, methyl testosterone, and testosterone propionate), androsterone was isolated in

the ketonic fraction of many specimens, appearing to be to some extent a "final, common end product" and indicating the existence of several modes of inactivation of androgenic steroids. The compound most active clinically when given *per os* is methyl testosterone, but it scarcely elevates the titers of urinary androgens. In contrast, testosterone propionate taken orally is ineffective; but it is absorbed, changed to androsterone, and excreted in the urine; bioassay of the urinary androgens gives extremely high values. Failure to isolate androsterone as a urinary end product following administration of dehydroisoandrosterone (a urinary compound in cases of masculinizing adrenal tumors), is worthy of note.—Authors' abstract.

FEINSTEIN, R. N., and F. E. STARE. [Univ. of Wisconsin, Madison, Wis.] THERMOSTABLE OXIDATIONS IN TUMOR AND MUSCLE TISSUE. Proc. Soc. Exper. Biol. & Med., 45:525-529. 1940.

Although minced Flexner-Jobling rat carcinoma has an aerobic respiration equal to that of rat skeletal muscle mince, the oxygen uptake in the presence of glutathione of thermostable powders (essentially denatured proteins) prepared from the tumor tissue is considerably lower than the oxygen uptake of similar powders prepared from muscle. This seems to parallel the fact that although the tumor powder and the muscle powder have approximately the same total sulfur content, the per cent of the total sulfur which is present as sulfhydryl sulfur is much lower in tumor powder than in muscle powder.—S. B-J.

GREENSTEIN, J. P., J. W. THOMPSON, and W. V. JENRETTE. [Nat. Cancer Inst., Bethesda, Md.] CHEMICAL STUDIES ON THE COMPONENTS OF NORMAL AND NEOPLASTIC TISSUES. III. THE COMPOSITION AND AMPHOTERIC PROPERTIES OF THE NUCLEOPROTEIN FRACTION OF THE JENSEN RAT SARCOMA. J. Nat. Cancer Inst., 1:367-376. 1940.

A study is being made of the nucleoproteins of the Jensen rat sarcoma in an effort to detect differences in the chemical properties of the constituents of neoplastic and normal tissues. An analysis of the nucleoproteins of the Jensen sarcoma reveals that the proportion of amino acids is similar to that previously reported for normal liver nucleoprotein. The minimum molecular weight of the nucleoprotein is about 40,000. In the native state it contains free –SH groups, and the number of these groups is increased by denaturation. Further studies have been carried out on the amphoteric nature of the nucleoprotein by potentiometric titration in 6 M guanidine HCL. Titration studies in water could not be done because of changing solubility throughout the pH range. It appears that the Jensen tumor nucleoprotein possesses considerable capacity to combine with acid and alkali, indicating a number of contained reactive groupings.—L. L. W.

GREENSTEIN, J. P., F. C. TURNER, and W. V. JENRETTE. [Nat. Cancer Inst., Bethesda, Md.] CHEMICAL STUDIES ON THE COMPONENTS OF NORMAL AND NEOPLASTIC TISSUES. IV. THE MELANIN-CONTAINING PSEUDOGLOBULIN OF THE MALIGNANT MELANOMA OF MICE. J. Nat. Cancer Inst., 1:377-385. 1940.

The proteins and melanin of mouse melanoma S-91 (Cloudman) are investigated in this paper. The proteins consist of over 99% pseudoglobulin (which contains nearly all the melanin), of about 0.05% euglobulin, and a trace of albumin. Analysis of the pseudoglobulin indicates a minimum molecular weight of about 20,000.

The melanin was separated from the pseudoglobulin by pancreatin digestion. It was found that protein-like material was still bound to the pigment, and that this was rich in sulfur-containing amino acids.

Viscosity experiments on the pseudoglobulin in water and in various denaturing salts indicates that in water the protein is of the globular type and is broken down into smaller more symmetrical fragments by the salts.

Chemical procedures are given in detail and a simple cellophane ultrafilter is described.—L. L. W.

HERSHBERG, E. B., and J. K. WOLFE. [Harvard Univ., Cambridge, Mass. and Peter Bent Brigham Hosp., Boston, Mass.] A RAPID EXTRACTOR FOR URINARY STEROIDS. J. Biol. Chem., 133:667-673. 1940.

A satisfactory apparatus is described for the rapid and efficient continuous extraction of steroids from urine. Heated carbon tetrachloride is passed through a sintered glass plate and allowed to fall in fine droplets through a long column of urine. The majority of the androgenic fraction of normal urines is extracted within ½ hour.—H. J. C.

HERSHBERG, E. B., J. K. WOLFE, and L. F. FIESER. [Harvard Univ., Cambridge, Mass.] POLAROGRAPHIC DETERMINATION OF CERTAIN NATURAL PRODUCTS. J. Am. Soc., 62:3516-3517. 1940.

Androsterone and dehydroisoandrosterone are converted into diketones by Oppenauer oxidation; during the process, the Δ^5 double bond of dehydroisoandrosterone is rearranged to the Δ^4 position thus forming the readily determinable α,β-unsaturated ketonic structure. Comparison of the polarogram obtained after oxidation of the ketonic fraction of a urinary extract from a female with an adrenal tumor with that made before the oxidation permits the determination of the amounts of dehydroisoandrosterone and androsterone in the extract. The results agree well with isolation experiments and with assays by colorimetric procedures.—H. J. C.

NORRIS, E. R., and E. E. TROESCHER. [Univ. of Washington, Seattle, Wash.] SYNTHESIS OF STEROL BY TUMOR-BEARING RATS. Cancer Research, 1:410-412. 1941.

Young rats bearing implanted Walker adenocarcinoma 256 and fed an adequate synthetic low-sterol diet consistently synthesized more sterol than normal litter mates, when the food intake of the latter was limited to conform to that of tumor-bearing litter mates.

Tumor-bearing rats excreted the same amount of sterol as normal control rats. The significantly greater sterol synthesis shown by tumor-bearing rats was entirely accounted for by their greater increases in body sterol.—Authors' summary.

SILBERBERG, M. and R. SILBERBERG. [Oscar Johnson Inst., St. Louis, Mo.] CHANGES IN CARTILAGE AND BONE OF IMMATURE FEMALE GUINEA PIGS DUE TO UNDERNOURISHMENT. Arch. Path., 30:675-688. 1940.

In these guinea pigs underfeeding causes atrophy of the cartilage cells and increased sclerosis of the matrix of the growth zone. Cartilage proliferation and ossification continue, but endochondral and periosteal bone formation are retarded. On refeeding, overproduction of cartilage cells and bone occurs at first; later, normal conditions are restored.—Authors' abstract.

WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] RETARDATION OF GROWTH OF THE RAT INGESTING p-DIMETHYLAMINOAZOBENZENE (BUTTER YELLOW). I. THE EFFECT OF VARIOUS DIETARY SUPPLEMENTS. J. Nat. Cancer Inst., 1:337-341. 1940.

Young rats of the Osborne-Mendel strain were placed on a basal diet with a relatively low protein content. They gained weight normally, however, and after a control period, 60 mgm. of butter-yellow (dimethylaminoazobenzene) was added to each 100 gm. of diet. This produced a definite retardation in growth. Again, after a control period, if l-cystine or dl-methionine (500 mgm. to 100 gm.) were added to the basal diet supplemented by butter-yellow, a prompt stimulation of growth occurred which was maintained. The addition of taurine, cysteic acid, or sodium sulfate to the basal diet containing butter-yellow failed to stimulate growth. The author suggests that butter-yellow probably produces a specific demand for a sulfur-containing amino acid, resulting in a limitation of the animal's ability to produce new tissue protein.—L. L. W.

WOLFE, J. K., E. B. HERSHBERG, and L. F. FIESER. [Harvard Univ., Cambridge, Mass.] THE POLAROGRAPHIC DETERMINATION OF KETONIC STEROIDS. J. Biol. Chem., 136:653-687. 1940.

This informative article describes the electro-chemograph and recording device, a newly designed dropping mercury electrode assembly, the necessary reagents, processing of the urinary extracts, experimental procedure, calibration of the apparatus and the analysis of the polarograms. Polarographic studies have been made on urinary extracts of normal males and females and on patients with Addison's disease, adrenal tumors, and various other pathological conditions. The method gives excellent analyses for a large number of steroids. The polarographic method has been used by other workers for the determination of α,β-unsaturated ketones such as testosterone, desoxycorticosterone and progesterone. By condensation with excess Girard's Reagent to give a derivative with a $=C=N-$ linkage, the determination of these hormones as well as corticosterone, Δ^4-cholestenone and 17-hydroxydehydrocorticosterone has been made specific and sensitive and it has become possible to determine accurately the total amount of 17-ketosteroids (androsterone, isoandrosterone, and dehydroisoandrosterone) in neutral urinary extracts even in the presence of companion substances. Under similar treatment, estrone may be determined whereas such saturated 3-ketosteroids as cholestanone and coprostanone are indifferent. The α,β-unsaturated Δ^4-3-ketosteroids may also be distinguished from Δ^1-3-ketosteroids.—H. J. C.

Immunology

CHEEVER, F. S. [Harvard Univ. Med. Sch., Boston, Mass.] A COMPLEMENT-FIXING ANTIBODY IN SERA OF RABBITS BEARING BROWN-PEARCE CARCINOMA. Proc. Soc. Exper. Biol. & Med., 45:517-522. 1940.

The antigen was prepared according to the method of Kidd. Brown-Pearce carcinoma, removed under aseptic precautions and freed of necrotic tissue, was ground in a mortar with gradual addition of isotonic saline to make a 1:20 suspension, allowed to stand overnight in the refrigerator, and then centrifuged at 3,800 r.p.m. twice for 5 and 20 minutes, respectively. The resulting slightly opalescent suspensions were inactivated for 30 minutes at 56° C. immediately prior to use. Complement fixation tests with this antigen were made with serums of rabbits bearing the Brown-Pearce carcinoma. Among 35 rabbits bearing Brown-Pearce carcinoma in various stages, positive complement fixation reactions were obtained with 5 specimens of serum.—S. B-J.

FURTH, J., and E. A. KABAT. [Cornell Univ. Med. Coll., New York, N. Y.] ASSOCIATION OF THE HETEROGENETIC ANTIGEN WITH A MATERIAL IN NORMAL AND TUMOR TISSUES SEDIMENTABLE AT HIGH SPEED. Science, 91:483-485. 1940.

There is in tissue extracts a material sedimentable at high speed with which are associated two distinct immunologically specific bodies. One of these is heat labile and species-specific; the other is heat stable and is identical with the heterogenetic (Forssman) antigen. Antiserums containing specific antibodies for each have been prepared. Experiments are in progress to determine whether the heavy species-specific substance and the Forssman antigen are distinct chemical entities or whether the Forssman hapten is part of the former.—Authors' abstract.

Leukemia

MURPHY, J. B., and E. STURM. [The Rockefeller Inst. for Med. Research, New York, N. Y.] THE TRANSMISSION OF AN INDUCED LYMPHATIC LEUKEMIA AND LYMPHOSARCOMA IN THE RAT. Cancer Research, 1:379-383. 1941.

A rat which had been injected in both groins with 1,2,5,6-dibenzanthracene in benzol developed typical lymphatic leukemia. The first lesions were noted in the regional lymph nodes where crystals of the agent were found imbedded in the tissue. It has proved possible to transfer the disease to other rats but the manifestations of the disease are varied depending on the site of injection of leukemia cells. When given intraperitoneally, the disease is manifested in the blood stream with involvement of only the lymph nodes and thymus. Subcutaneous inoculations with either blood cells or involved nodes with few exceptions produced local lymphosarcomas spreading only as far as the regional lymph nodes. Inoculation into the spleen produced leukemia with the principal manifestation in the spleen and liver leaving the superficial nodes and thymus untouched. The transmitted disease is described.—Authors' summary.

Miscellaneous

MENKIN, V. [Harvard Med. Sch., Boston, Mass.] DYNAMICS OF INFLAMMATION. AN INQUIRY INTO THE MECHANISM OF INFECTIOUS PROCESSES. Experimental Biology Monograph. 244 pp. Macmillan Co., New York, 1940.

This monograph reviews primarily the studies of the writer during the past 12 years, integrating, however, at the same time, his views with the findings and views of other investigators in the field of inflammation.—Author's abstract.

MENKIN, V. [Harvard Med. Sch., Boston, Mass.] MECHANISM OF LEUKOCYTOSIS WITH INFLAMMATION. THE NATURE OF THE LEUKOCYTOSIS-PROMOTING FACTOR IN EXUDATES. Arch. Path., 30:363-373. 1940.

There exists in inflammatory exudates a factor capable of inducing marked increase in the number of circulating

leukocytes. The presence of this leukocytosis-promoting factor is readily demonstrated by noting the effect of injected exudate on the leukocyte level of a normal animal. Various other unrelated materials, including blood serum, broth, turpentine, bacteria, nucleic acid derivatives, histamine, and leukotaxine, are ineffective in reproducing in dogs precisely the same pattern of response as produced by exudates. The leukocytosis-promoting factor bears no evident relationship to leukotaxine, the nitrogenous substance responsible for the increase in capillary permeability and leukocytic migration.

The leukocytosis-promoting factor is thermolabile and essentially nondiffusible. Observations on protein fractionation of exudates either after dialysis or after "salting out" of the material at various concentrations of ammonium sulfate indicate either that the leukocytosis-promoting factor is a globulin or that at least it is closely associated with the globulin fraction. The available data warrant the belief that the factor seems linked primarily with the pseudoglobulin fraction of inflammatory exudate.—Author's abstract.

MORRIS, H. P., and J. W. THOMPSON. [Nat. Cancer Inst., Bethesda, Md.] CONVENIENT INEXPENSIVE DEVICE FOR QUANTITATIVE HAND FEEDING OF MICE. J. Nat. Cancer Inst., 1:395-396. 1940.

The device described for hand feeding mice consists of an 18-gauge needle with an enlarged smooth tip of silver solder. The needle is bent to a convenient angle and is fitted to a tuberculin syringe.—L. L. W.

COMPARATIVE ONCOLOGY

JENNEY, F. S. [Univ. of Pittsburgh, Pittsburgh, Penn.] RETICULUM CELL SARCOMA OF THE RAT TRANSFERRED THROUGH TWELVE SUCCESSIVE PASSAGES IN ANIMALS OF RELATED STOCK. Cancer Research, 1:407-409. 1941.

Spontaneous reticulum cell sarcomas, arising in the mesentery at the ileocecal junction, were observed in a colony of Wistar stock rats. Ten of these tumors were transferred subcutaneously into animals of the same stock. Only one inoculated tumor survived for repeated passages. Among 116 inoculated rats there were about 42% successful takes through 12 passages. Microscopically, the tumor was composed of large pleomorphic cells, with one or more nuclei and a moderate amount of clear cytoplasm. Irregularly distributed among the cells was a reticulum of branching fibrils.—Author's abstract.

OLSON, C., JR. [Massachusetts State Coll., Amherst, Mass.] A TRANSMISSIBLE LYMPHOID TUMOR OF THE CHICKEN. Cancer Research, 1:413-421. 1941.

A transmissible lymphoid tumor of the chicken and the results of the first 30 serial passages are described. Transmission was accomplished by transplants of tumor tissue. Growth of the transplants occurred in 67.7% of those inoculated. Regression of implant occurred in 44.3% after development of growth. Metastasis to visceral organs occurred in 17% of chickens in which the implants developed. The implants developed better in young birds. No difference of resistance to growth of implants was observed in 3 stocks of chickens. The character of the tumor remained constant during serial passage.—Author's abstract.

Clinical and Pathological Reports

Diagnosis—General

PEET, M. N. [Univ. of Michigan, Ann Arbor, Mich.] VENTRICULOGRAPHY IN BRAIN TUMOR DIAGNOSIS. Surgery, 9:329. 1941.

The author uses a single posterolateral approach for ventriculography, rather than two trephines. Oxygen is injected. The value of this procedure is emphasized.—A. M.

STERN, K. [New York City Cancer Hosp., New York, N. Y.] INVESTIGATIONS ON THE RETICULOENDOTHELIAL FUNCTION OF CANCER PATIENTS. J. Lab. & Clin. Med., 26:809-817. 1941.

A modified Congo red test has been used to determine the reticulo-endothelial function, which was found to be impaired in 86% of cancer cases. This test cannot be used for diagnosis since the function is also impaired in many infectious diseases.—H. G. W.

Therapy—General

SCHMIDT, E. R., and MOHS, F. E. [Madison, Wis.] THE CHEMOSURGICAL TREATMENT OF CANCER, A MICROSCOPICALLY CONTROLLED METHOD. Surgery, 9:298. 1941.

Zinc chloride is used to fix tissue *in situ*. Following this, the tissue is excised and its base examined by immediate serial sections, with the aid of the microscope. Positive areas are retreated until a noncancerous layer is obtained. Four hundred and forty cases so treated were followed from 1 to $4\frac{1}{2}$ years. Ninety-three per cent were free of local recurrence.—A. M.

Skin and Subcutaneous Tissues

AMELANOTIC MELANOMA. CASE REPORT. [Waterbury Gen. Hosp. Waterbury, Conn.] Conn. State M. J., 5:193-196. 1941.

A case is reported in which metastases to the skin, tonsil, heart, mediastinum, omentum, and adrenals manifested themselves 15 years after the removal of a nonpigmented mole from the dorsum of the right second toe. Review of the original section revealed a nonpigmented mole with malignant transformation.—A. DeB.

DE CHOLNOKY, T. [The New York Post-Graduate Hosp., New York, N. Y.] MALIGNANT MELANOMA. A CLINICAL STUDY OF ONE HUNDRED SEVENTEEN CASES. Ann. Surg., 113:392-410. 1941.

This article reviews the results of surgical treatment in a small group of cases of melanoma. The youngest patient was 15 months old, the oldest 78 years. Only two cases were under 10. After 20 the distribution was similar for each decade, with 38% of the cases between 45 and 60. Forty per cent of the cases involved the head, 26% the lower extremities, and 15% the upper extremities. Regional lymph node involvement was present in 27.4% of cases at their first examination. Generalized metastases were present in 10%, yet only 1 case had an osseous metastasis. However, no organ is immune from metastases in this disease. No accurate relation could be established between the size of the primary lesion and regional lymph node involvement or general metastases. The relationship of

trauma, particularly chronic irritation and electric needle "therapy" to the development of melanoma from benign moles, is stressed. Metastases usually occur within 6 months to 2 years, though some lesions grow slowly for many years without dissemination. Many patients who survive 5 years harbor the disease. Metastases may appear as long as 27 years after surgery. Late distant metastases are seen most often after eye cases. Early radical excision followed by regional node dissection is advocated. Amputation is proposed for cases involving the foot, toes, or fingers. Of the 117 cases in this series, 75 were treated surgically; 36 cases lived 3 months to 13 years; 15 were lost to follow-up. Of the 24 dead cases, 16 died within 3 years and only 2 lived over 5 years. A total of only 26 cases were followed over 5 years. Of these, 9 are living and apparently well, 3 having survived node dissections for involved nodes. After 10 years 5 are well; only 1 had lymph node involvement. Four tables summarize the data. One lists the reported 5 year survivals in the literature. Eight photographs are included and a bibliography of 46 papers.—A. M.

HADLEY, H. G. [Washington, D. C.] TRAUMATIC EPITHELIAL CYSTS. Urol. & Cutan. Rev., 45:66. 1941.

Brief report of a case of epithelial cyst of the thumb, excised 36 years after original injury.—A. M.

TAYLOR, G. W., I. T. NATHANSON, and D. T. SHAW. [The Collis P. Huntington Hosp., Boston, Mass.] EPIDERMOID CARCINOMA OF THE EXTREMITIES WITH REFERENCE TO LYMPH NODE INVOLVEMENT. Ann. Surg., 113:268-275. 1941.

This report is based on 430 cases of epidermoid carcinoma of the extremities—284 involved the hand, 73 the leg, 41 the arm, and 32 the feet. The median age for cancer of the lower extremities (55) was 10 years less than for the upper (65). Forty-five per cent (193 cases) gave a history of a preexisting lesion. Chief of these were: senile keratosis (63), arsenical keratosis (14), injuries or scars (36), old burn scars (23), radiation dermatitis (10), chronic osteomyelitis (11), varicose ulcer (11), etc.

The local lesion was uncontrolled in about 10% of the 332 cases treated. Nine per cent of the finger cases, 15% of the hand cases, 26% of the arm cases, 41% of the foot cases, and 36% of the leg cases developed metastases. Although most of the lesions were large and of long duration, high grades were encountered in many on the arm and lower extremity. Leg carcinoma cases delayed therapy longer (2.8 years) than the remainder of the group (1.7 to 1.8 years) and 36% showed metastases when first seen. The popliteal nodes were involved in only 1 case. When nodes under 1 cm. were felt in the axilla, 11% were involved; in the epitrochlear region, 20% were involved; in the groin, 14% were involved. With nodes between 1 to 2 cm. these figures rose to 20%, 72%, 60%, and over 2 cm. to 86%, 100%, 94%. The margin for error is greatest in the axilla. Two upper extremity patients without involved nodes on admission subsequently developed nodes; only 1 patient who had a negative groin dissection later developed fatal recurrence in the groin. Forty-four axillary dissections were performed; 21 showed involved nodes, and 9 are living and well. Of 28 groin dissections, 14 showed disease and only 2 were salvaged. Roentgen therapy cured none. With upper extremity lesions axillary involvement increases with delay from 22% in 6 months to 75% in 3 years; in the lower extremity groin disease increases from 40% to 65%. None of the 11 cases with cancer arising in old osteomyelitis showed metastases. Only 10% of the upper extremity and 15% of the lower extremity fatal cases lived 2 years after therapy.

Delayed node dissection is advocated, reserving prophylactic dissections for large and high grade lesions. There are 6 tables and 2 references.—A. M.

Nervous System

SCHERER, H. J. [Bunge Inst., Antwerp, Belgium] CEREBRAL ASTROCYTOMAS AND THEIR DERIVATIVES. Am. J. Cancer, 40:159-198. 1940.

A complete pathological study of 18 cerebral astrocytomatous tumors occurring in a series of 125 necropsied gliomas is presented. Clinical summaries and macroscopic and histological descriptions in full are given. Certain salient facts appear from this study and a comparison of the literature. Histologically pure cerebral astrocytomas make up only about one-third of the total. Five such tumors were encountered in this material. Cerebral astrocytomas are primary, diffuse, uncircumscribed proliferations in contrast to the well circumscribed cerebellar astrocytomas. Microscopically, the tumor cells are less fibrillary in type in the cortex than in the white matter. Early tumors and those that have grown rapidly as evidenced by a short clinical course are less cellular than those of slow growth and long standing. Within the tumors pre-existing nervous parenchyma often persists and may be recognized. The astrocytomas furthermore are very subject to microcystic degeneration but rarely are areas of necrosis present. The vascularity of the tumors is slight and there is no evidence of angioplastic tendencies. The majority of astrocytomas are, at time of necropsy, partially transformed into typical glioblastomas. The reasons for this transformation are not clear, but the process is thought to be a dedifferentiation of the cells of a pure astrocytoma rather than the coexistence of 2 primary tumors arising from normal glia. The paper is supplemented with excellent photographs.—L. L. W.

STOOKEY, B., J. SCARFF, and M. TEITELBAUM. [New York, N. Y.] FRONTAL LOBECTOMY IN THE TREATMENT OF BRAIN TUMORS. Ann. Surg., 113:161-169. 1941.

The term "frontal lobectomy" is a misnomer as the operation involves the removal of the "prefrontal area," which is the frontal lobe exclusive of the motor, premotor, and speech areas. Previously, 7 cases of lobectomy with critical analysis were reported. This paper discusses the results of lobectomy, in 11 frontal lobe gliomas. There was no immediate mortality. Three patients with glioblastomas did poorly. In 6 of 9 cases with astrocytomas, removal seemed adequate enough to hope for cure. Removal of the diseased region results in a normal patient, as judged by psychometric and psychiatric determination. Seventeen papers are included in the bibliography.—A. M.

TRUEBLOOD, D. V. [King County Hosp., Seattle, Wash.] NEUROGENIC SARCOMA. Surg. Gynec. & Obst., 72:363-371. 1941.

Clinical study, with emphasis on the potential danger of benign neurofibromatosis.—H. G. W.

Female Genital Tract

KAZANCIGIL, T. R., W. LAQUEUR, and P. LADEWIG. [Univ. of Istanbul, Turkey] PAPILLO-ENDOTHELIOMA OVARII. REPORT OF THREE CASES AND A DISCUSSION OF SCHILLER'S "MESONEPHROMA OVARII." Am. J. Cancer, 40:199-212. 1940.

Three malignant ovarian tumors resembling Schiller's "mesonephroma ovarii" (Schiller, W. Am. J. Cancer, **35**: 1. 1939) are described. From the microscopic appearance and a study of plastic reconstructions, the authors conclude that the tumors are of endotheliomatous origin and do not represent tumors arising in mesonephric rests in the ovary.—L. L. W.

Intrathoracic Tumors—Lungs—Pleura

HALPERT, B., and B. PEARSON. [Louisiana State Univ. Sch. of Med.] THE CELLULAR STRUCTURE OF CARCINOMA OF THE LUNG. Am. J. Cancer, 40:213-218. 1940.

The cytology of 92 primary lung cancers occurring in a total of 7,433 necropsies was studied. Forty-seven were squamous cell, 17 were columnar cell, and 26 were the so-called "reserve cell" type. Some contained all three or two of the cell types but were classified according to the predominant one.

The figures of race and sex incidence of pulmonary carcinoma in this series are interesting. There were 48 cancers in 2,183 white males but only 5 in 987 white females. Thirty-eight of 2,618 colored males had pulmonary carcinomas but only one was found in 1,645 colored females coming to necropsy.—L. L. W.

LISA, J. R., C. SOLOMON, and E. J. GORDON. [City Hosp., Welfare Island, New York] LEUKEMOID REACTION IN CARCINOMATOUS SKELETAL AND SPLENIC METASTASES. Am. J. Cancer, 40:227-230. 1940.

A case of carcinomatosis with widespread skeletal and visceral metastases including the spleen is presented. The total white count was 28,450 with 60% lymphocytes or prolymphocytes. The platelets were 110,000. The patient before death showed a marked hemorrhagic tendency. At autopsy the tumor was found to be primary in a bronchus. The bone marrow was not unusual except where it was actually replaced by tumor metastasis.—L. L. W.

Gastrointestinal Tract

ADAMS, W. E. [Univ. of Chicago, Chicago, Ill.] RECENT PROGRESS IN THE SURGICAL TREATMENT OF CARCINOMA OF THE ESOPHAGUS. Surg., Gynec. & Obst., 72:312-317. 1941.

Discussion of surgical technic, which has so improved that whereas only 14 successful resections were reported up to 1934, during the next 4 years 15 cases were recorded, and since 1938 the author has collected 34 cases recorded or by correspondence. The 34 successful resections were made from 68 operations, and of the 34 cases 20 have lived as long as 4 years following operation.—H. G. W.

BRUNSCHWIG, A., T. H. CLARKE, J. VAN PROHASKA, and R. SCHMITZ. [University of Chicago, Sch. of Med., Chicago, Ill.] A GASTRIC SECRETORY DEPRESSANT IN EXTRACTS OF ACHLORHYDRIC CARCINOMATOUS STOMACHS. Ann. Surg., 113:41-46. 1941.

Searching for an inhibitory substance to account for achlorhydria in certain carcinomatous stomachs, the authors made extracts of such stomachs and observed the gastric secretion of dogs with pouch stomachs after intravenous injection of the extracts. Extracts were prepared from organs removed at autopsy or operation by grinding the neoplasm or entire musoca with sand, adding 50 cc. 0.2% HCl or distilled water per 12 gm. of tissue, boiling the mixture in a Kjeldahl flask for 10 minutes, permitting it to stay overnight at room temperature, centrifuging, and neutralizing the decanted supernatant fluid with NaOH. The extract is stored at 56° C. Since it contains a fine precipitate, it must be shaken before use.

Dogs with gastric pouches were fed meat until a vigorous flow of acid was obtained. The inhibition of pouch secretion and free acid was observed in many 30 to 120 minutes after injection of the extract. Three or more positive experiments in different dogs were used as a criterion for activity of an extract. One hundred and fifty-six experiments were performed on 22 achlorhydric carcinomatous stomachs; 11 (50%) showed a secretory depressant. Of carcinomatous stomachs without achlorhydria 25% (3 in 12) possessed this inhibitory factor, but so did 16% of stomachs without cancer, 24% of stomachs in patients with extragastric malignancy and 12% of carcinomatous colons. Achlorhydria could be maintained by repeated injections of inhibitory extract from any source. Fever, atropine, urine, concentrated glucose, and normal tissue extracts may produce transitory inhibition of gastric secretion in these pouch dogs. A chart, 3 tables, and 7 references are included.—A. M.

COLLER, F. A. [Ann Arbor, Mich.] REGIONAL LYMPHATIC METASTASES OF CARCINOMA OF THE COLON. OBSERVATION ON CLARIFIED SPECIMENS OF COLON AND ATTACHED MESENTERY. Surgery, 9:318-319. 1941.

The author's method made it possible to demonstrate 52 lymph nodes per specimen, whereas the usual methods reveal 14. Twenty-eight of 46 cases (61%) showed metastases. Often, no correlation existed between the size or the duration of the primary cancer and the presence or the extent of metastases. Metastases seemed to parallel the grade of malignancy.—A. M.

GORDON, W. C. [Metropolitan Hosp. New York, N. Y.] PRIMARY MELANOMA OF THE SMALL INTESTINE. Rev. Gastroenterol., 8:36-44. 1941.

Eight cases of apparently primary melanomas of the small intestine are recorded in the literature, to which is added a ninth.—H. G. W.

HOTSLEY, J. S. [Richmond, Va.] RESECTION OF THE DUODENUM FOR TUMOR OF THE AMPULLA OF VATER. Surgery, 9:321. 1941.

A modification of the Whipple procedure is described. It was employed to resect a "fibroadenoma of the ampulla."—A. M.

HUNT, V. C. [Los Angeles, Calif.] HEMANGIOMA OF THE LARGE BOWEL. Surgery, 9:300. 1941.

A case is reported with involvement of the entire rectosigmoid, rectum, and uterus. Removal was accomplished by a two stage abdominoperineal resection of rectum and uterus. Forty-eight cases have been reported—20 involved the colon and 28 involved the rectum. Pathologically they varied from "capillary nevi" to cavernous hemangiomas.—A. M.

JACKSON, J. A. [Jackson Clinic, Madison, Wis.] TOTAL GASTRECTOMY FOR LINITIS PLASTICA. Am. J. Surg., 51:414-418. 1941.

Report of a case of successful removal of a diffuse carcinoma of the stomach by total gastrectomy.—H. G. W.

JOHNS, F. S. [Richmond, Va.] RECURRENT CARCINOMA OF THE RECTUM. Surgery, 9, 318. 1941.

A case report of a male with local recurrence 15 years after a Kraske operation for rectal cancer. The patient was alive and well at 42, 1 year after reoperation.—A. M.

MOENE, I. [Bergen, Norway] A CASE OF VON RECKLINGHAUSEN'S DISEASE ASSOCIATED WITH GANGLIONEUROMA OF THE STOMACH. Surgery, 9:150. 1941.

A case report of a 54-year-old man alive and well $1\frac{1}{2}$ years after the removal of a ganglioneuroma the size of an orange from the lesser curvature.—A. M.

MULLEN, T. F. [Univ. of California Med. Sch. San Francisco, Calif.] SOME FACTORS INFLUENCING THE CURABILITY OF CANCER OF THE STOMACH. Surg., Gynec. & Obst., 72:298-306. 1941.

Clinical lecture, with emphasis on the importance of the skillful surgeon.—H. G. W.

PEARLMAN, S. [Chicago, Ill.] SARCO-CARCINOMA OF THE ESOPHAGUS. IS THERE SUCH AN ENTITY? Surgery, 9:143. 1941.

The author reviews 17 reported cases of so-called carcinosarcoma of the esophagus and 8 of his own. He demonstrates that anaplastic squamous cell carcinomas often contain spindle cells and doubts whether there is any such entity.—A. M.

RASMUSSEN, R. A., and A. BRUNSCHWIG. [Univ. of Chicago Sch. of Med., Chicago, Ill.] PEPTIC ACTIVITY OF ACHLORHYDRIC HUMAN GASTRIC JUICES FROM CARCINOMATOUS STOMACHS. COMPARATIVE STUDY. Proc. Soc. Exper. Biol. & Med., 46:298-300. 1941.

Mett's tubes, 1.0 mm. in diameter were filled with egg albumin. They were then placed in boiling water until the protein coagulated and filed into 2 cm. lengths for storage until used. All samples of gastric juice were obtained from empty stomachs after overnight starvation by the usual histamine test for gastric secretory function. Tests were made as follows: 5 cc. of the juice was brought, if necessary, to pH 1.2 (thymol blue indicator) with normal HCl. The juice was then placed in a 30 cc. beaker, 2 Mett's tubes immersed in it, the beaker covered with a watch glass, and the whole incubated at 37.5° C. for 24 hours. The results were recorded in term of millimeters of albumin digested at each end of the 2 tubes used.

Gastric juice was obtained from patients with, (1) achlorhydria due to gastric cancer, pernicious anemia, or induced by x-radiation to the stomach; (2) achlorhydria without the last 3 types of causes, (3) carcinomatous stomachs secreting free acid, and (4) patients with acid gastric juice due to a variety of other conditions. The results show that achlorhydric juices contain substantially less pepsin than those containing free HCl. In this series, the low pepsin content of achlorhydric gastric juices from cancerous stomachs was of the same order as that from achlorhydric juices from other sources.—M. B.

REIMANN, S. P. [Lankenau Hosp. Research Inst., Philadelphia, Penn.] ON SOME PATHOLOGICAL ASPECTS OF CARCINOMA OF THE RECTUM. Rev. Gastroenterol., 8:24-28. 1941.

General considerations.—H. G. W.

STOREY, C. F. [Med. Corps, U. S. Navy] TUMORS OF THE VERMIFORM APPENDIX. U. S. Nav. M. Bull., 38:324-357. 1940.

The author reports 6 cases including a questionable primary carcinoma, a carcinoid, a mucocele, an extension from a cecum carcinoma, a metastasis from a bronchogenic carcinoma, and a tuberculous granuloma.

Tumors of the appendix, mostly benign, are not rare. They usually cause few or no symptoms, but may simulate chronic appendicitis. It is the author's impression that about 1 in 400 appendices (0.25%) subjected to routine study are either carcinoid or carcinoma. Carcinoid is probably ten times as common as carcinoma. Many appendiceal carcinomas probably represent extension from the cecum. Ages of patients with these tumors vary from 5 to 92 years. Carcinoids are most common in the 3rd and 4th decade. The youngest patient with authentic adenocarcinoma was 14 years old. Carcinoids, situated at the tip in 90% of cases, rarely recur after appendectomy. No metastatic case of appendiceal carcinoid has been reported. Adenocarcinomas are of the gelatinous variety and occur about the base. They may become generalized. Mucoceles occur in about 0.2% of all appendices seen at postmortem. Two varieties are distinguishable: 1) the "hydrops" type, with a dilated lumen and atrophic mucous membrane, 2) the true "mucocele" type, with hyperplastic and even papillary mucosa. The latter is similar to papillary cystadenoma of the ovary. Rupture may cause pseudomyxoma of the peritoneum and in such cases x-ray therapy should follow surgery.

Sarcomas (lymphosarcoma, myxosarcoma, and myosarcoma) have been reported. Plexiform neuroma of the appendix is common microscopically. Neuromatous overgrowth is often seen in association with the obliterated appendix. Rare benign tumors are: intra-appendiceal polyp, fibroma, myoma, myxoma, and hemangioma. The bibliography contains 51 references.—A. M.

WALTERS, W. [Rochester, Minn.] A REVIEW OF CASES OF CARCINOMA OF THE STOMACH AT THE MAYO CLINIC SINCE 1908. Surgery, 9:297. 1941.

Twenty-five per cent of these cases gave an "ulcer-type" history; 80% received relief from a medical regimen for ulcer; and 58% of all cases were subjected to surgery. About one-half (26%) were resected. Of the resected cases, 25% lived 10 years; 10% lived 20 years. The survival curve for resected cases after 6 years parallels the expected mortality curve of that age. Recurrence after 6 years is uncommon.—A. M.

Bone and Bone Marrow

ABBOTT, W. D. [Mercy Hosp., Des Moines, Ia.] ANGIOMA OF THE SKULL. Ann. Surg., 113:306-311. 1941.

The author lists 26 cases of angioma of the skull reported in the literature. They vary in age from 4 months to 76 years. Only 13 hemangiomas are recorded in the Bone Tumor Registry's 1,831 bone neoplasms; 3 of these involve the skull. In 9 cases tumor was successfully removed. The author reports on 2 cases observed by him. One, a 3-year-old girl with a right frontal capillary hemangioma, is well 4 years after removal of the tumor. The second, a boy of 3, is well $1\frac{1}{2}$ years after removal of a right parietal cavernous hemangioma. Twenty-seven

papers are included in the bibliography. There as 7 photographs.—A. M.

ALUSTIZA, F. [Ramos Mejía Hosp., Buenos Aires] TUMORES PRIMITIVOS Y MALIGNOS DE LOS HUESOS. [PRIMARY MALIGNANT TUMORS OF THE BONES.] Rev. Asoc. méd. argent., 54:381-387. 1940.

A report of 5 cases illustrated by x-ray pictures.—M. D-R.

LEUCUTIA, T. [Harper Hosp., Detroit, Mich.] BENIGN GIANT CELL TUMORS OF BONE. LATE RESULTS IN 33 CASES. Surgery, 9:327. 1941.

Eighteen cases were treated by irradiation therapy only. On the basis of end results this is judged the best method. Surgery holds the disadvantage of infection. Escharotics act as irritants and delay healing. Healing is most rapid with roentgen ray therapy. The patients received 6 to 8 series of treatments over a 2-year period. Using 200 kv., 525 r were given as an initial series and decreased 10% each time.—A. M.

WALDRON, C. W. [Univ. of Minnesota, Minneapolis, Minn.] TUMORS OF THE UPPER JAW. Surg. Gynec. & Obst., 72:503-511. 1941.

General review, not suitable for abstracting.—H. G. W.

WALKER, W. B. [Charlotte Hungerford Hosp., Torrington, Conn.] OSTEOGENIC SARCOMA: A CASE REPORT. Conn. State M. J., 5:43-44. 1941.

This report of a case of osteochondrosarcoma emphasizes the occasional difficulty in diagnosing malignancy of bone tumors by biopsy and x-ray.—A. DeB.

WILSON, H. [Memphis, Tenn.] EXTRASKELETAL OSSIFYING TUMORS. Ann. Surg., 113:95-111. 1941.

The author lists 30 cases of malignant bone-forming tumors of the soft parts from the literature. Twelve were in the breast and 7 in the thyroid. He briefly lists 10 cases, from the Bone Tumor Registry and the Univ. of Chicago, including 7 malignant extraskeletal osteogenic sarcomas and 3 benign osteomas. One of the latter occurred in a neurofibroma associated with Von Recklinghausen's disease. Heterotopic ossification is discussed. The article is illustrated with roentgenograms and photomicrographs. The bibliography includes 36 authors.—A. M.

Muscle and Tendon

HYMAN, M. A. and M. LEDERER. [The Jewish Hosp., Brooklyn, N. Y.] FIBROSARCOMA OF THE DIAPHRAGM. Arch. Path., 31:204-210. 1941.

Case report with brief review of the 17 cases in the literature.—H. G. W.

LEICHNER, W., and A. SCHAEFER. [Mt. Sinai Hosp., Hartford, Conn.] SYNOVIAL SARCOMA: A CASE REPORT. Conn. State M. J., 5:113-115. 1941.

A case of synovial sarcoma in a child 10 years old is reported, the pathology of this condition discussed, and its high malignancy emphasized.—A. DeB.

Leukemia, Lymphosarcoma, Hodgkin's Disease

CRAMER, H. [Rudolf Virchow Krankerhaus, Berlin] DIE LEUKAMIE ALS TUMOR-PROBLEM. [LEUKEMIA AS A TUMOR PROBLEM.] München. med. Wchnschr., 48:1326-1333. 1940.

Report of a case of chronic myelogenous leukemia with premenstrual exacerbations uninfluenced by irradiation therapy. In another case, severe leukopenia followed pyramidon therapy. All therapeutic methods failed, including transfusion and x-ray to the long bones, yet an immediate response was obtained with 50,000 I. U. of progynon B. Hormone excretion assays were made on the urine of 18 leukemia cases. The data suggested a high prolan output and some parallelism, between the clinical course of leukemia, not the blood count, and the prolan output. Nine cases of Hodgkin's disease showed no hormone changes. Of 32 collected tumors in males, 5 showed an increased prolan excretion, including 1 seminoma. Of 33 tumors in females 8 cases showed an increase in urinary prolan; 6 of these occurred in 15 breast tumors.—A. M.

MILLER, F. R., and W. A. HAUSE. [Jefferson Med. Coll., Philadelphia, Penn.] SPECIFIC SUBSTANCES IN THE URINE OF LEUKEMIA PATIENTS. Proc. Soc. Exper. Biol. & Med., 45:387-388. 1940.

By a modified McCullagh's method, details of which are given, specific substances were extracted from the urine of patients with various types of leukemia. Extracts of urine in cases of chronic myeloid leukemia, chronic lymphoid leukemia, and acute lymphoblastic leukemia into guinea pigs caused the animals to become emaciated. After death the animals presented myeloid proliferations in the liver, spleen, adrenal, lung, and kidney. Guinea pigs injected with extracts from acute lymphoblastic leukemia showed amaturation of the bone marrow. Lymphadenopathy and lymphocytic proliferation of the liver occurred in guinea pigs injected with extracts from urine in lymphoid leukemia. The saponifiable and nonsaponifiable fractions of the chloroform residue are being tested.—S. B-J.

Adrenal

CAHILL, G. F. [New York, N. Y.] ADRENAL TUMOR AND THE USE OF AIR INSUFFLATION IN DIAGNOSIS. Surgery, 9:330. 1941.

Air insufflation of the pararenal fascial space was employed by the author in 200 cases without accident.—A. M.

Pancreas

KENNARD, H. E. [Massachusetts Gen. Hosp., Boston, Mass.] PAPILLARY CYSTADENOCARCINOMA OF THE PANCREAS. Surgery, 9:65-79. 1941.

The author reports in detail the 3rd case of malignant cystic tumor in the pancreas to occur at the Massachusetts General Hospital in 20 years. The Mayo Clinic reported only 4 malignant cases in 88 pancreatic cysts. Case reports collected from the literature are reviewed and diagnostic and therapeutic measures discussed. References are given to 49 papers.—A. M.

KRIEG, E. G. [Woman's Hosp., Detroit, Mich.] HETEROTOPIC PANCREATIC TISSUE PRODUCING PYLORIC OBSTRUCTION. A REVIEW AND CASE REPORT. Ann. Surg., 113:364-370. 1941.

Three hundred and forty cases of heterotopic pancreatic tissue were reported in the literature up to June, 1939. Autopsy statistics indicate an incidence of close to 2%. About one-third of the cases show Langerhans islets. The chief sites for such tissue are: stomach (31.46%), duodenum (31.83%), jejunum (21.7%), and ileum (9.36%). Cases have been reported in the mesentery, biliary tract, spleen, umbilicus, appendix, and a dermoid cyst. The size may vary up to $6 \times 3.5 \times 1$ cm. In approximately one-half of the cases this pancreatic tissue occurs in the submucosa of the intestine, but may involve any or all layers. Most cases are asymptomatic. Where symptoms do appear they are not characteristic. All the pathological changes noted in the normal gland may occur in heterotopic pancreas,

including necrosis, malignancy, and ulcer. In close to 40% a diagnosis of peptic ulcer was made. Pyloric obstruction occurred in 17 cases. It is the result of muscular hypertrophy and spasm due to the heterotopic tissue. The author presents such a case in a 45-year-old woman. Four tables summarize some features of the literature. There is a bibliography of 23 authors.—A. M.

ORR, T. G. [Kansas City, Mo.] RESECTION OF THE HEAD OF THE PANCREAS FOR CARCINOMA. Surgery, 9:296. 1941.

Report of a case. Previous cholecystectomy necessitated a choledochogastrostomy. Three weeks later the head of the pancreas and duodenum were removed. The patient returned after 6 weeks with a pancreatic fistula. Lipocaic is suggested for use in these cases.—A. M.

Thyroid

GEER, W. A. [Bridgeport Hosp., Bridgeport, Conn.] TUMORS OF LATERAL ABERRANT THYROID TISSUE. Connecticut M. J., 4:670-671. 1940.

Two cases of surgically treated lateral aberrant thyroid tumor are reported.—A. DeB.

SMITH, M. K. [St. Luke's Hosp., New York, N. Y.] PAPILLARY ADENOMA OF ABERRANT THYROID. Ann. Surg., 113:77-81. 1941.

The patient, a 24-year-old male, had bilateral firm cervical masses of 10 months duration. They suggested enlarged lymph nodes, but biopsy showed "papillary carcinoma." Two courses of roentgen therapy brought no relief. A series of 3 operations were performed, at 2-week intervals beginning 6 months after the original biopsy, removing masses from the right side of the neck, the isthmus, the right thyroid lobe, the mediastinum, and the left side. All showed papillary adenoma of aberrant thyroid. A photograph is shown. One paper is quoted.—A. M.

TOUROFF, A. S. W. [Beth Israel Hosp., New York, N. Y.] COEXISTING CARCINOMATA OF THYROID AND ABERRANT THYROID WITH REGIONAL METASTASES. Ann. Surg., 113:73-76. 1941.

The author presents the case of a 23-year-old female who had unexplained fever for 4 weeks after subsidence of pneumonia. Painless, movable, globular masses extended from mastoid to clavicle along the anterior border of the right sternomastoid. The right lobe of the thyroid was enlarged. Biopsy, taken of this slowly growing mass which had been present in her neck for 12 years, showed "papilloma" of an aberrant thyroid. Three years before a diagnosis of tuberculous cervical lymphadenitis had been made. Two months before admission an abscess in the mass, which followed an injection in that area, was drained, and biopsy showed only "inflamed necrotic tissue." The lobe was widely resected and the masses removed. The diagnosis was "papillary adenocarcinoma of thyroid and aberrant thyroid, metastasizing in lymph nodes." Postoperative x-ray therapy was administered. The patient is alive and well 14 months after operation.—A. M.

Miscellaneous

DARÚ, E., and B. BOCANDIO. [Rivadavia Hosp., Buenos Aires] SEUDOMIXOMA PERITONEAL. [PSEUDOMYXOMA OF THE PERITONEUM.] Rev. méd.-quir. de pat. fem., 15:288-297. 1940.

Report of a case.—M. D-R.

FLYNN, J. M. [Rochester, N. Y.] RETROPERITONEAL LIPOSARCOMA WITH MYXOMATOUS DEGENERATION. Radiology, 35:90-92. 1940.

Case report with x-ray illustrations.—E. A. L.

HARBERT, F. [Med. Corps, U. S. Navy] MALIGNANT MELANOMA OF THE ORBIT WITH CASE REPORT. U. S. Nav. M. Bull., 38:378-380. 1941.

A 42-year-old Samoan female had an exenteration performed for a huge tumor protruding from the orbit and invading the lids. It began as a protuberance from the pupillary body 6 months before. She is living and well after 3 years. Biopsy showed the polyhedral cell type of melanoma. The paper includes 2 photographs.—A. M.

LEVY, J. H., and E. R. PUND. [Univ. of Georgia Sch. of Med., Augusta, Ga.] PRIMARY SARCOMA OF OMENTUM. Am. J. Cancer, 40:219-226. 1940.

Two cases of primary sarcoma of the omentum are presented fully and an additional one as an addendum. The tumors apparently arose from endothelium although the sections suggested a possible lipoblastic origin as well. Fat stains were negative. A review of such cases in the literature revealed that in the majority, operative removal did not prevent recurrences. In one of the authors' cases, a total of 1,750 r given in divided doses had no apparent effect on the course of the tumor.—L. L. W.

LISA, J. R., L. HIRSCHHORN, and C. A. HART. [New York City Hosp., New York, N. Y.] TUMORS OF THE HEART. Arch. Int. Med., 67:91-113. 1941.

Report of 4 cases of metastatic tumors of the heart causing cardiac dysfunction, with a review of the literature. The outstanding feature has been intractable failure without obvious cause, and sudden death is frequent, especially in the case of primary tumors. The appearance of cardiac failure or arrhythmia in association with a known malignant growth should lead to a consideration of the possibility of cardiac metastasis.—H. G. W.

ROHDENBURG, G. L. [Lenox Hill Hosp., New York, N. Y.] THE GLUCOSE TOLERANCE TEST IN ITS RELATION TO CANCER. Cancer Research, 1:311-312. 1941.

No relationship between the degree of malignancy and glucose tolerance in individual cases could be established in a study of 128 cases over a period of 6 years. If group behavior is considered, it appears to be true that groups having the more anaplastic tumors do not rid the blood stream as quickly of an excess of glucose as do groups carrying the less anaplastic tumors. This assertion is based upon a consideration of group averages. The glucose tolerance of the tumor bearer furnishes no data of prognostic value as to the expectancy of life of the tumor bearer.—Author's summary.

Statistics

ANNUAL REPORT OF THE CHIEF INSPECTOR OF FACTORIES FOR THE YEAR 1939. H. M. Stationery Office, 1-57. 1941.

This report contains a chapter upon industrial diseases by the Senior Medical Inspector, Dr. John C. Bridge, in which the following instances of occupational cancer occur. The preparation of this report has been delayed by war conditions.

1) In a fatal case of arsenical poisoning due to sodium arsenite there was pigmentation of the trunk and limbs, warty growths all over the body, perforation of the nasal

septum, and a primary cancer of the right lung with metastatic growths in adjacent glands and in the liver.

2) "Papilloma of the bladder. Inquests were held in 2 cases of men employed in the manufacture of synthetic dyes. In one case a history of previous substantial contact with beta-naphthylamine and benzidine for 13 years was elicited, but in the other no more definite information was available than that the patient had been in contact with various intermediate dye products for at least 11 years prior to retirement 1 year before."

3) Three cases of cancer of the nose and 5 cases of cancer of the lung were observed in nickel workers. Full accounts of this form of cancer are given in previous reports.—E. L. K.

CIOCCO, A. [U. S. Public Health Service] ON THE MORTALITY IN HUSBANDS AND WIVES. Human Biol., 12:508-531. 1940.

The author analyzes the death records of 2,571 white married couples both of whom died in Washington County, Maryland during the period 1898-1938. "The material originates from official vital statistics records of all the dead white persons of the community, and no intentional selection has been made relative to the two main variables" dealt with in the study, *viz.*, "age at death and cause of death." Two significant findings result from this investigation. The first is the "high positive correlation in the length of life of husbands and wives." The second is the apparent "tendency for husbands and wives to die from the same cause when one of the spouses dies from either tuberculosis, influenza and pneumonia, heart disease, or cancer. This tendency is statistically significant with respect to all four causes mentioned." "When the husbands died from cancer, 15.5% of their wives also died from this cause, but when the husbands died from other causes, only 10.3% of the wives died from cancer. Similar results are obtained when the frequency of cancer in the husbands of women who died from cancer is compared with that in the husbands of women who did not die from cancer." The paper includes 5 tables and 2 graphs.—G. DeB.

DUFFIELD, T. J., and M. DI MARIO. [Dept. of Health, New York, N. Y.] CANCER MORTALITY IN NEW YORK CITY: 1901 TO 1938. Cancer Research, 1:413-421. 1941.

The mortality from cancer in the City of New York for the years 1901 to 1938, inclusive, has been analyzed by age, sex, and site of primary involvement. After eliminating the effect of the ageing of the population through the process of standardization, it is shown that the death rate from recorded cancer increased 73% in the period under review. In the same period of time, the mortality charged to tuberculosis fell almost 80% and the death rate from pneumonia declined more than 70%.

Although in the early years of this experience the *recorded mortality* from cancer was much higher among women, the standardized cancer death rate among males was higher than that among females in 5 of the last 8 years covered by this study. Cancer mortality among males exceeds that among females except in the period of childbearing and in the following decade of life (25 to 54 years), when the death rates are relatively low.

It is only in the higher ages that the recorded mortality from cancer has shown any increase. This fact, in conjunction with the finding that, with the exception of the female breast, significant increases in the recorded cancer mortality in recent years have been limited to internal sites (generally inaccessible for diagnosis), leads to the suggestion that much of the apparent increase in cancer mortality has resulted from improved diagnostic technics and increased opportunities to employ them, rather than from a large increase in cancer incidence.—Authors' abstract.

SCIENTIFIC SOCIETIES AND RESEARCH ORGANIZATIONS

FIFTEENTH MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Connecticut M. J., 4:208-211. 1940.

Details of establishing a successful tumor clinic in a general hospital are presented.—A. DeB.

SIXTEENTH MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Connecticut M. J., 4:338-350, 1940.

Carcinoma of the ovary and sarcoma of the uterus are discussed. Papers on the care of inoperable cancer from the medical and neurosurgical points of view are presented.—A. DeB.

SEVENTEENTH MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Connecticut M. J., 4:454-470. 1940.

Papers are presented on the problems and errors in the diagnosis of malignant tumors of the central nervous system, of the respiratory tract, of the kidney, of the uterus, and of the stomach. The administrative phases of the state tumor program are outlined.—A. DeB.

EIGHTEENTH MEETING OF THE ASSOCIATION OF CONNECTICUT TUMOR CLINICS. Conn. State M. J., 5:197-206. 1941.

The program included papers on the following subjects: pathology of benign tumors of the breast, a statistical study of cancer of the breast, radical mastectomy, simple mastectomy, radiation therapy of carcinoma of the breast, and treatment of recurrences and metastases in cancer of the breast.—A. DeB.

PROCEEDINGS OF THE SEVENTH INTERNATIONAL GENETICAL CONGRESS. [Edinburgh, Scotland. 23-30 August 1939] Edited by R. C. Punnett. Cambridge Univ. Press, 1941.

Section I of the Congress was devoted to the genetical aspects of growth, and the *Proceedings,* issued as a supplementary volume of the *Journal of Genetics,* include abstracts of papers on genetic constitution and the origin of different cancers (L. C. Strong, N. Dobrovolskaia-Zavadskaia, M. R. Curtis and W. F. Dunning, G. M. Bonser); the selection of genetic material for the experimental study of cancer (H. J. Bagg, H. B. Andervont); dominance in spontaneous cancer (P. A. Gorer); tumors in twins (M. T. Macklin); transplantation and differentiation (P. A. Gorer); the connection between brown degeneration of the adrenals and mammary cancer (W. Cramer and E. S. Horning, L. Kreyberg, G. M. Bonser, R. C. Bamber); the genetics of growth and differentiation (E. B. Ford); and related subjects.

In another section, papers by W. T. Astbury, D. Crowfoot, T. Caspersson, H. H. McKinney, and J. W. Gowen, forming part of a symposium on protein and virus studies in relation to the problem of the gene, also contain material of interest to the student of cancer.—A. H.